EFFECT OF GENTCHROMAN ON OVULATION AND OVARIAN SIZE

THESIS

FOR

MASTER OF SURGERY

(OBSTETRICS & GYNAECOLOGY)

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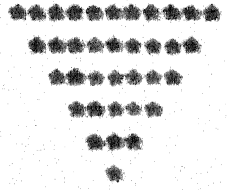

C E R T I F I C A T E

This is to certify that the work entitled "EFFECT OF CENTCHROMAN ON OVULATION AND OVARIAN SIZE" which is being submitted as a thesis for Master of Surgery (Obstetrics and Gynaecology) by Dr. Manjari Chadha has been carried out under my direct supervision and guidance. The observations recorded have been periodically checked and verified by me.

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I N T R O D U C T I O N

INTRODUCTION

For centuries Man had a desire to exercise voluntary control over conception, to plan it according to his need and wish. This led to the development of various contraceptive methods. Contraception has been practised in one form or the other throughout history. It allows the partners of an expression of their sexual needs without incurring the risk of unwanted pregnancies. Contraception has great importance in the field of preventive medicine; being essential to the health of individuals, families and community.

Attention was focussed on a simple and effective oral chemical contraceptive and this search culminated in the development of the oral contraceptive pill. It began towards the end of the nineteenth century when a number of investigators including Beard (1897) and Zschokke (1898) observed that in various animal species ovulation did not occur in an ovary with a well developed corpus luteum. The sex hormones (estrogens and progesterones) and their property of inhibiting ovulation was discovered. Parkes and Bellerby in 1926 and later Burdick and Pincus observed that pregnancy could be prevented in rats and mice by injecting them with estrogens within one or two days after mating. Similar effect was observed with oral estrogens.

The oral pills (estrogen and progesterone combination) were introduced in the early 1960's. During the 1970's following their widespread use their side effects and difficulties in mass administration became apparent. The search for an alternative oral contraceptive began.

The designing of molecules possessing estrogenic, antiestrogenic (atypical estrogens) and antiprogestational properties which would possibly interefare primarily with infraovarian events appeared a possible approach to the problem. Diphenylethylenes, Triphenylethylenes, Methoxybenzefuran and Naphthofuran derivatives were shown to possess marked antifertility activity. However owing to their side effects they were soon deleted from further studies. Later on 3,4 diphenylchromenes and 3,4-diphenylchromans were synthesized and their properties studied. The transform of the chromans were shown to possess significant antifertility action, whereas the cis form was devoid of any such activity. One of the trans chromans is centchroman.

The chemical name of centchroman is 3,4 trans-2,2 - dimethyl -3, Phenyl-4- (p- (β- Pyrrolidinoethoxy)-7-methoxychroman. The compound possesses estrogenic, antiestrogenic and antiprogestogenic properties. It has been

shown to possess contraceptive properties as a post coital pill as well as an oral contraceptive. The chemical formula of this compound is C_{30} H_{35} O_3 N- Hcl; with a melting point of 165 - 166°C. The t 1/2 is 169 hours. The compound is stable even after storage upto 3 years. The trans isomer is centchroman and it has a high order of antifertility efficacy, while the corresponding cis-isomer is devoid of any of these activities.

The post coital antifertility activity of centchroman was described by Kamboj, Chandra, Kar, Ray, Grover and Anand (1971). The compound was found to possess weak estrogenic, antiestrogenic and anti-progestational properties (Kamboj et al 1971).

Post coital administration of centchroman as a single oral dose within 24 hours of coitus was able to prevent pregnancy in rats, mice (1.25 mg/kg), dogs and rhesus monkey (2.5 mg/kg) and also intramuscularly in dogs (1.5 mg/kg). The antifertility effect was promptly reversible.

Acute toxicity studies on centchroman in rats and monkeys have shown that the compound has a very high margin of safety. It is devoid of any pharmacological effect which may be reflected as a side effect during clinical use. It has also shown its safety in chronic toxicity studies in rats and monkeys.

The spectrum of pharmacological actions of centchroman includes antiinflammatory activity, incomplete - adrenergic blockade, non specific spasmolytic activity and mild anorexigenic effect at higher dose levels than the contraceptive dose. Clinical pharmacology studies in healthy human female volunteers also showed the compound to be safe (H. Chandra et al 1977).

The site of action of centchroman in rats and monkeys seemed to be at the level of fallopian tubes and uterus. It causes arrest of egg development. The transport, fertilisation and viability of the ova is not disturbed. It appeared that the antifertility effect was probably by interfering with the action of estrogen and progesterone. It prevented implantation and arrested development of ova.

Studies done in human females show that centchroman activates the hypothalamo-pituitory axis and causes an increase in serum gonadotrophin levels. The antiestrogenic effect is exerted differentially on vagina, cervix and endometrium. The contraceptive effect is thought to be mainly due to the action on cervical mucous and endometrium affecting sperm transport and implantation (Rama Vaidya et al 1977). Centchroman appears to exert its antifertility action by means of its multiple hormonal effects such as estrogenic, antiestrogenic and antiprogestational activity. Besides it also has effect on ova development and all these properties make it a potent contraceptive.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

By the end of the Nineteenth Century Beard (1897), Zschokke (1898) and a number of other investigators observed that in various animal species ovulation failed to occur in an ovary which contained a well developed corpus luteum. During the first three decades of the Twentieth Century further evidence was accumulated indicating that extract of corpus luteum was capable of inhibiting ovulation (Pearl and Surface 1914, Haberlandt 1921, Smith 1929, Parkes 1929). In 1934 four independent groups of workers were successful in isolating the corpus luteum hormone-progesterone (Butenandt et al 1934, Slotta et al 1934, Allen and Wintersteiner 1934, Hartmann and Wellstein 1934). Following its isolation the physiological actions of progesterone were studied intensively. It became apparent that the hormone was capable of inhibiting ovulation in a variety of animal species (Makepeace et al 1937, Astwood and Fevold 1939).

Simultaneously work on estrogens was also in progress. It was observed that pregnancy in mice and rats could be prevented by injecting them with estrogen within a day or two of mating (Parkes and Bellerby 1926, Burdick and Pincus 1935).

In 1938 work by Parkes and Dodds showed that oral estrogens are also capable of preventing pregnancy. However these observations were not given much importance at that time; perhaps because the attention was not yet focussed sharply on the problem of population growth. In the early 1960's the oral contraceptive pill (the estrogen, progesterone combination) was introduced. During the first decade of their use attention was focussed on the benefit of pregnancy prevention and risk of abnormal cycle bleeding. During the 1970's following their widespread use the adverse effects of oral contraceptives became apparent.

Attention was now focussed on the development of newer oral contraceptives. Since the demonstration of the estrogenic activity of diphenylethylenes by Dodds et al in 1938 and estrogenic/antiestrogenic activity of triphenyl-ethylenes (Segal and Nelson 1958) it became clear that estrogenic activity is not highly structure specific. 2-phyenyl -3 (p (β-pyrrol idinoethoxy) phenyl)-6- methoxy-benzofuran and related compounds like 2- phenyl -3- (p-(β- pyrrol idinoethoxy) phenyl)- naphthofuran were found to possess significant antifertility activity when given within 24 hours of coital act. They showed varying degree of estrogenic and antiestrogenic activity. Owing to their

side effects they were deleted from further studies.
A variety of related structural types were synthesized and their properties studied. These included the chromenes and the chromans. Among the chromenes (3,4 - diphenylchromenes) it was observed that introduction of alkyl group at 2 position decreased the antifertility activity. The trans forms of the 3,4 diphenyl chromans were in general more active than the corresponding cis compounds. It was suggested that this difference in biological activity is due to the change in the confirmation of molecules which has important implication in receptor binding.

Trans-2, 2- dimethyl -3- phenyl -4- p- (β- pyrrolidinoethoxy) phenyl -7- methoxychroman (centchroman) was synthesized. This compound was shown in experiments to prevent pregnancy in rats, mice, dogs and rhesus monkeys. Various pharmacological actions of this compound have been studied.

1. <u>Antifertility efficacy</u> :

Oral administration of centchroman at doses of 0.25 mg/kg on day 1-5 post coitum causes 100% prevention of pregnancy in rats and mice. Single administration of centchroman to rats at doses of 1.25 mg and 2.5 mg/kg on

any one of the days 1-4 post coitum causes 100% pregnancy prevention. The litter size was reduced upto 55-62% at both the doses. At 1 mg/kg dose there was 100% prevention of pregnancy when administered on any one day of days 1-3 post coitum. On day 4 it was partially effective (60% prevention) and on day 5 it was ineffective. 0.25 mg/kg dose was virtually ineffective. Thus 1.25 mg/kg is the minimum effective dose in the single day post coitum regime.

In mice also a single feeding of centchroman (1.25 mg/kg) was 100% effective in preventing pregnancy when given on day 1, 2 or 3 post coitum.

In dogs centchroman 2.5 and 5 mg/kg orally once a day or intramuscularly (1.5 and 2.5 mg/kg) 24 hours after mating caused 100% prevention of pregnancy and there was no evidence of implantation.

In rhesus monkeys also 2.5 mg/kg oral centchroman on the day following the coital act caused cent per cent prevention of pregnancy during a eight month trial. Discontinuation of the treatment caused prompt return of fertility (V.P. Kamboj et al 1977).

The post coital (60 mg) and weekly schedule of 120 mg and 60 mg dose in women of reproductive age group (20-35 years) has provided acceptable pregnancy rate. This being 4-5 pregnancies in 100 women years of use in weekly dose schedule.

2. Hormonal properties :

(a) Estrogenic activity : In immature rats administration of centchroman by oral (0.1, 0.25, 0.5, 1.25 and 2.0 mg/kg) or by subcutaneous route (1, 5, 10, 50 and 100 µg/animal) caused a significant increase in uterine weight. By oral route centchroman showed 40-50% uterotrophic activity as compared to estrone and only 23-44% by subcutaneous route. There was no indication of a dose response relationship. At 1.25 mg and 2.0 mg/kg by oral route and 50 and 100 ug by subcutaneous route vaginal opening was noted and the smears showed a proestrus and/or estrus condition. Same effects were observed in immature rhesus monkeys too. The uterotrophic potency was 64% that of estrone. The uterotrophic activity reached its peak two days after the administration of centchroman or estrone, declined thereafter and became virtually nil by 15 days (V.P. Kamboj et al 1977).

However in another study on immature mice oral administration of centchroman ranging from 5-200 µg (total dose) produced a linear increase in uterine weight. Similar dose related increase in uterine weight was observed by administration of ethinyl estradiol and mestranol.

The estrogenic effect of centchroman as assessed by vaginal cornification in ovariectomised mice after oral administration was 8-10 times less than that of mestranol

and ethinyl estradiol. Thus at the dose levels studied centchroman exhibited estrogenic activity both in uterotrophic and vaginal cornification test. (S.R. Munshi, R.K. Nair and P.K. Devi, 1977). Although its effect on the uterus is as rapid as that of estrone it has a latent period in causing vaginal cornification (V.P. Kamboj et al, 1977).

(b) Antiestrogenic properties : Estrone (1 µg/animal) when given to immature female rats as oral or injectable preparation causes increase in uterine weight and vaginal cornification (estrus type vaginal smears). Simultaneous administration of centchroman (0.125 mg to 5 mg/kg) by oral or parenteral route also caused a significant increase in uterine weight. However it was less than that caused by estrone alone. This also caused suppression of vaginal cornification. Thus centchroman demonstrated antiuterotrophic action.

Centchroman is relatively more potent in inhibiting uterotrophic activity of estrone than in preventing estrone induced vaginal cornification (V.P. Kamboj et al, 1977).

However in delayed implantation test the number of implantations was not affected after concurrent administration of progesterone (6 mg, subcutaneous), estradiol

dipropionate (1 ug, subcutaneous) and centchroman (0.25 mg/kg, oral) from days 8-12 of pregnancy. Centchroman thus exhibited no antiestrogenic activity in this test. Centchroman perse did not induce implantation in this assay (V. P. Kamboj et al, 1977).

Rama Vaidya and coworkers (1977) in human studies have shown a distinct antiestrogenic effect on vaginal cytohormonal pattern at 60 mg/week and 120 mg/week doses. The antiestrogenic effect was also reflected in the cervical mucous score at 120 mg/week dose, but was not so noticeable at 60 mg/week dose.

(c) Progestational and antiprogestational activity :

In 1977 Kumari et al reported that centchroman did not accelerate either the metabolism or the elimination of progesterone in the target tissues of ovariectomized rats. Centchroman also failed to decrease the availability of progesterone in the target tissues.

In Clauberg assay norethisterone administered orally at different dose levels produced a graded response on the rabbit endometrium. Administration of centchroman failed to induce a similar proliferation thus reflecting absence of progestational activity at that dose level. However the same dose of centchroman when administered alongwith norethisterone was able to only partially inhibit

endometrial proliferation suggesting a weak antiprogestational activity. Centchroman at a dose of 25 µg/day was not able to maintain pregnancy in ovariectomized rats thus indicating absence of progestational activity. (R.K. Nair, T.A. Sheyte, S.R. Munshi 1977).

Work by S.N. Roy and J.K. Dutta (1977) supported the view that centchroman is devoid of antiprogestational property. Adult female rats were ovariectomized on third day of pregnancy and were treated with progesterone for 15 days. During last 5 days of progesterone therapy 1 mg/kg of centchroman was given daily. The results showed that centchroman failed to counteract the increase of weight and the total content of biochemical constituent of uterus; caused by progesterone therapy.

Centchroman administered with or without progesterone on day 4 of pseudopregnancy to intact, traumatized mice caused complete inhibition of decifuoma formation, thus confirming its antiprogestational activity. The antiprogestational activity was however marginal. These results confirmed the earlier observations of Kamboj et al (1971).

3. Endocrine Pharmacology :

(a) Effect on pituitory : Centchroman showed no effect on weight and gonadotrophin content of young male rat pituitory (V.P. Kamboj et al, 1977).

Dutta and Roy (1977) reported a gonadotrophin inhibitory effect of centchroman, but at higher dose levels this is being counteracted by some other property which facilitates release of gonadotrophins.

They however in 1980 reported a gonadotrophin releasing action of the compound in animals as well as in humans. The presumed stimulation of the secretion of gonadotrophins may be partly to the antiestrogenic action of the compound and partly to its direct positive feedback action on LH secretion.

Centchroman modulates serum level of gonadotrophin in human as well as in rats. Centchroman augments the release and/or synthesis of gonadotrophins from the pituitory acting at the level of the hypothalamus (S. N. Roy, G.L. Kumari and others, 1976).

Centchroman was found to induce ovulation in anovulatory women and stimulate LH secretion in gonadal dysgenesis patients with or without estrogen therapy. It has also been reported to stimulate LH secretion in normal male volunteers (S.N. Roy et al, 1977).

Rama Vaidya et al in 1977 reported increased pituitory gonadotrophin synthesis, storage and release in healthy female volunteers treated with centchroman.

In man there was distinct rise in serum and urinary levels of LH after 7 days of centchroman treatment (A.R.Sheth et al, 1977).

(b) Effect on thyroid :

Centchroman (1.25 mg/kg oral x 5 days) had no significant effect on thyroid weight, ^{131}I uptake in immature female rhesus monkeys (V.P. Kamboj et al, 1977).

(c) Effect on adrenal :

Studies in male rats have shown increase in relative weight of adrenal gland in the drug treated group. The increase in relative adrenal weight may represent true adrenal hypertrophy (R.P. Das, Somnath Roy, G.L. Kumari, 1977).

Centchroman (1.25 mg/kg oral x 5 days) did not influence the excretion rate of 24 hour urinary 17-OH-KGS in immature female rhesus monkeys whereas estrone caused a slight increase.

(4) Effect on fetus and fertility of the offspring :

Centchroman given orally (1.25 and 2 mg/kg) on days 5-7 of pregnancy to rats had no effect on the developing

blastocyst or the newly implanted fetus. However the number of blastocysts implanted was 17-32% less than that of controls.

A single feeding of centchroman on day 8 of pregnancy caused 30% fetal loss as against 14% in controls. No adverse effect on the genital organs of the fetus or neonates was revealed by histologic examination. No teratogenic effects were reported. The fertility of the offsprings showed no detrimental effect in next two generations.

Thus centchroman was seen to cause some fetal resorption in rats when administered peri or post implantation. No abnormal genital development or teretogenicity was found in the fetus and their postnatal sexual development and subsequent fertility potential remained unimpaired (V.P. Kamboj et al, 1977).

(5) Mechanism of action :

The mechanism of action of non steroidal antifertility agents in the female has been investigated and the general concept is that the antiestrogen binds to the cytosol estrogen receptor sites thereby displacing estrogen, thus causing a lowering of the effect of estradiol (Ghosh and Roy, 1977).

The action of endogenous estrogen might be blocked at the receptor level by centchroman competing for the same binding sites. Recently it has been shown that pretreatment

with centchroman inhibited in vivo uptake of labelled estradiol by hypothalamus, uterus, fallopian tubes, adrenal glands and liver (U.M. Joshi, V.K. Naik and P.S. Susheela, 1977).

Centchroman inhibits the pregnancy changes in uterus. It thus appears that interaction between centchroman and endogenous hormones might play a role in inhibition of decidual cell reaction. Lowering of lactic acid and glycogen concentration in uterine fluid of treated animals may be an important factor in contraceptive action since blastocyst was unlikely to survive in milieu depleted of these things (V.P. Kamboj, M.M. Singh and A.B. Kar, 1973).

(a) Effect on ova :

Centchroman administered orally (1.25 mg/kg) on day 1 of pregnancy to rats did not impede transport and fertilization of ova. There was however arrest of development in about 30% of ova.

Thus a high percentage of blastocysts (normal or degenerating) could be collected from the uterus of centchroman treated animals on day 6, whereas barely a few blastocysts were recovered at this time in controls. Apparently the bulk of them had implanted.

(b) Effect on deciduoma formation :

Centchroman (1.25 mg/kg) on day 1 of pregnancy prevented deciduoma formation in tubectomized, ovariectomized and traumatized rats treated with progesterone (2 mg daily) for 3 days.

In controls the traumatized horn was significantly heavier than its contralateral horn and that of centchroman treated animals and showed massive decidual swellings.

(c) Effect of estrogen and progesterone on antifertility action :

A single feeding of centchroman (1.25 mg/kg on day 1 of pregnancy) caused 100% prevention of implantation in rats. Progesterone (5mg/rat) given to compound treated animals from days 1-5 of pregnancy failed to induced implantation. Estradiol dipropionate per se did not induce implantation in compound treated animals. However progesterone administered in combination with estradiol dipropionate induced high percentage of implantations.

Thus centchroman interfered with the action of both the estrogen and progesterone since neither of these hormones per se could induce implantation in compound treated rats.

(d) Change in uterine mileu :

The administration of centchroman decreased significantly potassium concentration ofuterine fluid on day 5 of pregnancy in rats. Antiimplantation action of centchroman may be due to decrease in potassium concentration of uterine fluid on 5th day of pregnancy which thereafter may increase negative membrane potential on endometrium. With the result a negative charged endometrium repelled blastocyst bearing a similar charge and thus prevented implantation (Anand, O. Prakash, S.K. Roy, 1981).

As already mentioned, lowering of lactic acid and glycogen concentration in uterine fluid of treated animals may be an important factor in contraceptive action since blastocyst is unlikely to survive in mileu deplected of these things (V.P. Kamboj, M.M. Singh and A.B. Kar, 1973).

The antifertility action of centchroman seems to be at the level of both the fallopian tube and the uterus since there is some arrest of egg development and marked inhibition of deciduoma formation. Centchroman appears to exert its antifertility effect by interfering with the action of both estrogen and progesterone (V.P. Kamboj, B.S. Setty, Harish Chandra, S.K. Roy and Amiya, B. Kar, 1977).

A study done over human female volunteers showed that centchroman may have its contraceptive effect mainly due

to its action over cervical mucous and endometrium affecting sperm transport and implantation (R.A. Vaidya, U. Joshi, P. Meherji, N. Rege, S. Betrabet, L. Joshi, A. Sheth and P.K. Devi, 1977).

(6) Toxicity :

The pharmacological profile of centchroman showed antiinflammatory activity, incomplete alpha adrenergic blockade, non specific spasmolytic activity and a mild anorexigenic effect at high doses (S.S. Mukerjee, N. Sethi, G. N. Srivastava, A.K.Roy, S. Nityanand and S.K. Mukherjee,1977).

Chronic toxicity :

Centchroman was administered orally at doses of 6.25, 12.5 and 25 mg/kg once daily for seven months to young adult male and female albino rats. It did not show any evidence of toxicity. Similar study was also carried out in rhesus monkey with various doses. Hematology as well as biochemistry and histopathology of different organs did not reveal any adverse effect. Thus centchroman was shown to be devoid of any toxicity upto twenty times the contraceptive dose (S.S. Mukerjee, N. Sethi, G.N. Srivastava, P.S. Roy, S. Nityanand and S.K. Mukherjee, 1977).

S.S. Mukherjee, N. Sethi and others in 1977 observed that antiinflammatory activity will be no impediment to the

use of centchroman as a contraceptive. Moreover this effect is observed at a very much higher dose than the contraceptive dose.

Centchroman possesses an excellent therapeutic index and is devoid of any pharmacological effect which may be reflected as a side effect during clinical use. Chronic toxicity studies on monkeys and rats showed that leucocyte counts and haematological values were within normal limits of variation. The blood chemistry consisting of blood sugar, blood urea, SGPT showed normal values, within limits of variation. Centchroman may affect the metabolic function of liver after prolonged administration. It has been reported that oral contraceptives and other non steroidal estrogens can increase liver weight particularly at high doses which is considered to be due to non specific stress reaction(Mukerjee et al, 1977).

Acute toxicity :

This was determined by giving graded dose of compound to groups of mice and rats. LD_{50} was determined by intraperitoneal route in mice and by oral route both in mice and rats. LD_{50} was 400 mg/kg by intraperitoneal route. Oral contraceptive dose in mice and rats is 1.25 mg/kg. Since the oral LD_{50} was more than 1500 mg/kg, the compound had a very high margin of safety and at this dose mortality rate was 20%.

It was devoid of any significant gross effects or CNS effects. The anorexigenic activity observed at only high dose was unlikely to be a drawback in clinical dose range (I.M. Chak, P.R. Dua, K. Kar, R.C. Srimal and B.N. Dhawan, 1977).

(7) Teratogenicity :

Oral administration of centchroman to pregnant mice and rabbits at doses of 20, 40 and 80 times of 100% antifertility dose (ED_{100}) during period of organogenesis did not cause any abortion or congenital anomalies. There were no defects in skeleton and different organs of fetus. The resorption rate in mice was within normal limits. Only 50 mg/kg, the higher dose showed a higher rate that was 26%. In rabbit it is 40% as against 12-14% in controls of both the species. The litter size and weight in both species were comparable to control. No abnormality developed in offspring during postnatal growth upto 6 weeks in both animals (N. Sethi, 1977).

(8) Clinical pharmacology studies :

Clinical pharmacological evaluation of centchroman in normal healthy human volunteers was undertaken to determine the maximum tolerated dose and to find out any abnormal toxic effects in humans.

H. Chandra, R.C. Srimal, V.P. Kamboj, B.N. Dhawan and N.N. Gupta in 1977 conducted a single dose tolerance

study in 40 volunteers. Upto a dose of 320 mg centchroman was well tolerated without any side effects. In multiple dose schedule lasting 30 days 28 volunteers participated. The compound was found safe at doses of 60 and 120 mg/day. Laboratory tests were normal in all the groups. There was no significant change in the vital signs like pulse, blood pressure and respiration at even the highest dose employed. From the animal data reported by Chak et al a decrease of blood pressure could be expected due to non specific spasmolytic activity of the compound, but in none of the volunteers such a reduction was observed.

In one of the volunteers menstruation was delayed by 30 days and in another there was scanty bleeding lasting 1 day only during the medication month. Symptoms recorded were headache, letharginess, nausea, bodyache, giddiness, fever and burning micturition.

Another study was carried out by Rama Vaidya et al in 1977 on 10 healthy women of child bearing age with history of normal menstrual cycles. Centchroman was given at 60 and 120 mg weekly dose. Increase in the cycle length was noticed in all cases. This was probably due to the lengthening of follicular phase. They concluded that centchroman at 120 and 60 mg/week dose schedule does not seem to inhibit

ovulation although it may have its contraceptive effect mainly due to its action on cervical mucous and endometrium affecting sperm transport and implantation.

S.N. Roy et al (1977) carried out a study on the effect of centchroman administration in normospermic and oligospermic individuals. In both normal and oligospermic cases there was no alteration in liver and kidney function tests during drug therapy.

MATERIAL AND METHODS

MATERIAL AND METHODS

The present study was carried out in the Department of Obstetrics and Gynaecology, Department of Pathology and Department of Biochemistry, Maharani Laxmi Bai Medical College and Hospital, Jhansi during one year study period.

1. SELECTION OF CASES :

The study group comprised of cases attending the out patient department of the Department of Obstetrics and Gynaecology. Normal healthy women of reproductive age group (20-35 years) with normal clinical and gynaecological history were registered after obtaining their informed consent.

Inclusion criteria :

(a) Volunteers should be having normal menstrual cycle pattern.

(b) Post abortion cases would be enrolled after at least one normal cycle.

Exclusion criteria :

(a) Pregnant or lactating women were excluded from the study.

(b) History of recent jaundice and severe anaemia, diabetes mellitus, hypertension or any other major illness.

(c) Women taking steroidal contraceptives for at least 3 months prior to enrolment.

Women included in the study were not allowed to use other methods of contraception during the study period.

History :

All the patients were in the reproductive age group (20-35 years) with normal menstrual history. Complete obstetrical and menstrual history was taken.

General Examination :

Thorough general examination of the patient was done and pulse, blood pressure, pallor, edema and weight of the patient was noted.

Systemic Examination :

Examination of cardiovascular system, respiratory system and abdomen was done.

Local Examination :

(i) Per speculum examination (P/S) - It was done to inspect the cervix and vaginal wall.

(ii) Per vaginum examination (P/V) - It was done to note the position and size of the uterus and its appendages.

The ovaries were examined to note any enlargement and whether palpable or not palpable.

2. DOSAGE SCHEDULE :

Centchroman 30 mg tablets, oral, twice a week for the first three months and then once a week schedule was followed. Patients were instructed to take 30 mg tablet on the first day of ensuing menses after registration and thereafter every Sunday and Wednesday irrespective of menses day. The tablets were continued even if the subsequent menses were delayed. From the 4th month onwards the patients were asked to take one tablet (30 mg) on every Sunday irrespective of the menses day.

3. CRITERIA OF CONTRACEPTIVE EVALUATION :

The volunteers were asked to follow the schedule of drug use strictly. Any lapse on volunteer part was recorded.

The contraceptive efficacy was determined from the number of method failure (MF) pregnancies during the study period.

Any pregnancy occuring due to tablet omission or non-schedule use was classified as patient failure (PF).

4. FOLLOW UP :

Clinical observations and findings of laboratory investigations were recorded before drug administration and subsequently at periodic intervals (Post drug).

Laboratory tests :

Following tests were carried out before administration of drug (0) and subsequently at +3, +6, +12 months of drug intake.

(i) Urine examination for albumin and sugar
(ii) Haemoglobin (g/dl)
(iii) Serum bilirubin (mg/dl)
(iv) SGPT (I.U.)
(v) Blood urea (mg/dl)
(vi) Serum creatinine (mg/dl)
(vii) Serum lipids :
  (a) Cholesterol (mg/dl)
  (b) Triglycerides (mg/dl)

Urine albumin :

Presence of proteins in urine was determined by heat coagulation test.

Urine sugar :

Qualitative determination of sugar in urine was done by Benedict reagent.

Haemoglobin :

It was estimated by Sahli's Haemoglobinometer. A definite quantity of blood is converted into acid hematin by the addition of N/10 Hcl. Its colour is then matched against the standard provided in the haemoglobinometer.

Serum bilirubin :

It was determined by the Van Den Berg Method (1913). The method is based on the fact that in soluble form bilirubin reacts with the diazo reagent and forms a purple red colour which is measured colorimetrically.

SGPT :

It is based on the principle that Ketoglutaric acid and aspartic acid when treated together with serum get converted to glutamic acid and pyruvic acid by enzymatic action. This is treated with 2-4-dinitrophenyl hydrazine in alkaline medium which gives brown coloured hydrazone which is measured colorimetrically.

Blood Urea :

This is determined by the Nesslerization method. The principle is that urea is converted to ammonium carbonate by the action of enzyme urease. The protein free filtrate is

treated with Nessler's reagent. The ammonia liberated reacts with it and forms a yellow colour compound which is measured colorimetrically.

Serum creatinine :

This is determined by modified version of Brod et al's method (1948). Creatinine reacts with picric acid in alkaline medium and a red colour develops which is measured colorimetrically. The reaction is not specific but at least over 85% colour is due to creatinine.

Serum lipids :

Serum total cholesterol and serum triglycerides were estimated with standard diagnostic kits :-

(a) Serum Total Cholesterol : This was estimated by commercial chemical kits supplied by Ethnor.

The basic principle is that cholesterol reacts with hot solution of ferric perchlorate, ethyl acetate and sulphuric acid and gives a lavender coloured complex which is measured colorimetrically (Wyambenga and Pillegi method, 1970).

(b) Serum triglycerides :

It was estimated by acetyl acetone method chemical kits supplied by Span. The principle behind is that triglycerides are determined by measuring glycerol after its liberation from fatty acids by saponification. Glycerol is oxidised by sodium metaperiodate to formaldehyde which is directly proportional to the amount of triglyceride.

Special tests :

(i) Vaginal smear examination
(ii) Cervical mucous study
(iii) Ultrasonography

Vaginal smear examination :

Vaginal smear was prepared on 14th/15th and 21st day of pretreatment cycle and every treatment cycle. The smear was studied for evidence of Trichomonal and Monilial infection.

The superficial cells, intermediate cells and parabasal cells were studied and the maturation index and the karyopyknotic index was determined.

Trichomonas vaginalis :

They are frequently present in vaginal smears and their presence is not associated with hormonal factors.

In smears stained by Pap's stain they are seen as small, shapeless usually pale grey spots of variable size scattered between the exfoliated cells.

Monilia :

This is a fungi related to yeast which attacks the glycogen rich epithelium. In vaginal smears oval or round spores and mycelia may be seen. The mycelia are seen as irregular granular threads (hyphae) of variable length appearing pale in colour.

Superficial cells :

They are large, delicate, polyhedral cells with sharply defined cell borders which may be irregular of indented. The nucleus is pknotic. The cytoplasm may be eosinophilic or cyanophilic.

Intermediate cells :

These are medium sized cells with extreme variability in size. The cytoplasm is mostly cyanophilic. The nuclei are large and vesicular.

Parabasal cells :

These are rounded cells with cynophilic cytoplasm and the nucleus is rounded and large with distinct structure.

Maturation Index :

It expresses the level of cellular maturation attained at the time of exfoliation. Two hundred cells are counted in different fields and the number of superficial cells, intermediate cells and parabasal cells is determined. This is expressed in terms of 100 cells.
M.I. = Parabasal cells/Intermediate cells/Superficial cells.

Karyopyknotic Index :

Karyopyknotic index (KPI) denotes the percentage of cells with nuclear pyknosis. Two hundred cells are counted and those with pyknotic nuclei are expressed in percentage.

Smear fixation :

The vaginal smear was taken from the lateral vaginal wall before any gynaecological examination. It was prepared from secretions by spreading on two clean glass slides. The slides were immediately transferred to a mixture containing equal parts of 95% alcohol and ether.

Staining :

The Papanicolaou's staining method was used. This technique includes the following steps and the slides were passed through the following solutions in sequential manner :

1- 80% alcohol (1/2 min)

2- 70% alcohol (1/2 min)

3- 50% alcohol (1/2 min)

4- Distilled water (1/2 min)

5- Harris Haematoxylin (3 min) - Nuclear staining

6- Distilled water (1/2 min)

7- 0.25% aqueous Hcl (6 dips)

8- Running water (6 min)

9- Distilled water (1/2 min)

10- 50% alcohol (1/2 min)

11- 70% alcohol (1/2 min)

12- 80% alcohol (1/2 min)

13- 95% alcohol (1/2 min)

14- Orange G-6 ($1\frac{1}{2}$ min)

15- 95% alcohol (1/2 min) | Separate containers

16- 95% alcohol (1/2 min) |

17- EA-15 ($1\frac{1}{2}$ min) (Eosin Azure)

18- 95% alcohol (1/2 min) |

19- 95% alcohol (1/2 min) | Separate containers.

20- 95% alcohol (1/2 min) |

21- Absolute alcohol (1/2 min)

22- Xylol alcohol (1/2 min)

23- Xylol (1/2 min)

Finally mounting was done in DPX.

The nuclei are stained with Harris haematoxylin and the cytoplasm with Orange G and alcoholic light green - Bismarck Brown Eosin (Polychromatic stain) solution.

Cervical mucous study :

Cervical mucous was studied for Spinbarkeit and Fern test on 14th/15th and 21st day of cycle in the pretreatment cycle and then subsequently in each treatment cycle.

Spinbarkeit test :

A drop of cervical mucous was put on slide and was covered with another slide. Both the slides were slowly separated and the distance, upto which the two slides could be separated without breaking the mucous was noted in centimetres.

Fern test :

A drop of mucous was spread on a glass slide washed in distilled water to make a thick smear and was allowed to dry. It was examined under low power to see the ferning pattern.

Ultrasonography :

Ultrasonography was done to measure the ovarian size by Real Time Sector Scanner employing a frequency of 3.5 MHz (Philips S.D.R. 1550 XP).

The ultrasound transducer has piezo electric crystals. When an electric current is passed through the crystal a pulse of ultrasound is produced. This is passed through tissues and is reflected back in different intensities by different tissues depending upon their density. The echoes are converted back into electrical impulses in the transducer and are then perceived on a screen as images. The images are displayed in different shades of grey. Cystic areas appear dark and more solid areas appear whiter.

Ultrasound scanning is carried out with the patient supine and the urinary bladder physiologically distended to provide an accoustic window into the pelvis. Overdistension of the bladder is avoided.

Serial longitudinal and transverse sections are taken. The transducer is angled towards the side wall of the pelvis. The ovaries are mobile organs and are suspended from the dorsal surface of the broad ligament by the mesovarium. The internal iliac vessels are seen posteriorly.

The size of the ovary is variable depending upon the phase of the menstrual cycle and endocrinological status. The maximum length is usually less than 4 cm although occasionally thin ovaries may be longer.

An estimation for ovarian volume was done using the ellipsoid formula.

Vol. in c.c. = Longaxis X short axis X A.P. axis X 0.5.

O B S E R V A T I O N S

OBSERVATIONS

In the present study we have studied the effect of Centchroman a new non steroidal oral contraceptive on ovulation and ovarian size in the females of reproductive age group (20-35 years). Besides the effect on various biochemical parameters and side effects were also noted.

During first visit of the subject to the department thorough general examination was done. Basal sample of blood was withdrawn for various biochemical tests as mentioned earlier (Basal sample). The basal ovarian size was noted by ultrasonography. Vaginal cytology and cervical mucus study was done in the desired phase (14th day and 21st day of menstrual cycle). The woman was given the drug according to the schedule and the blood and urine tests and ovarian size measurement was done at +3, +6 and +12 months. Vaginal cytology and cervical mucus study was done in the appropriate phase in each cycle.

The observations made are mentioned in the form of various tables.

A : GENERAL CHARACTERS :

TABLE - I

Distribution of cases according to age (Maximum age recorded was 34 years).

S.No.	Age group (In years)	Number of cases	Percentage
1.	20 - 22	3	16.67
2.	23 - 25	7	38.89
3.	26 - 28	5	27.77
4.	29 - 31	1	5.55
5.	32 - 34	2	11.11
	Total	18	

Seven cases (38.89%) were in the age group 23-25 years, 5 cases (27.77%) belonged to age group of 26-28 years, 3 cases (16.67%) belonged to the age group of 20-22 years, 2 cases (11.11%) were in 32-34 years age group and one case (5.55%) was in 29-31 years age group.

TABLE - II

Distribution of cases according to parity

S.No.	Parity	Number of cases	Percentage
1.	1	3	16.67
2.	2	7	38.89
3.	3	3	16.67
4.	4	2	11.11
5.	5 and above	3	16.67
	Total	18	

Seven cases (38.89%) were second para, 3 cases (16.67%) each were with a parity of one, three and five and above. 2 cases (11.11%) were fourth para.

TABLE - III

Distribution of cases according to Initial (0) cycle duration.

S.No.	Cycle duration (days)	Number of cases	Percentage
1.	Upto 24	0	0
2.	25 - 35	18	100.00
3.	36 - 45	0	0
4.	45 and more	0	0
	Total	18	

All the cases had a cycle duration of 25-35 days.

TABLE - IV

Distribution of treatment cycles according to cycle duration.

S.No.	Cycle duration (days)	Number of cycle	Percentage
1.	Upto 24	3	2.68
2.	25 - 35	92	82.14
3.	36 - 45	12	10.71
4.	45 and more	5	4.46
	Total	112	

In 82.14% cycles the cycle duration was 25-35 days. In 10.71% cycles the cycles were prolonged for 36-45 days. 4.46% cycles were of a duration of 45 days or more. The cycle length was reduced to 24 days or less in 2.68% of cycles.

TABLE - V

Distribution of cases according to duration of flow in pretreatment cycle.

S.No.	Duration of flow (days)	Number of cases	Percentage
1.	2 - 3	8	44.44
2.	4 - 5	8	44.44
3.	6 or more	2	11.11
	Total	18	

Table V shows 8 cases (44.44%) each were having a duration of flow of 2-3 and 4-5 days. 2 cases (11.11%) were having a duration of flow of 6 days or more.

TABLE - VI

Distribution of treatment cycles according to duration of flow.

S.No.	Duration of flow (days)	Number of cycle	Percentage
1.	2 - 3	85	75.89
2.	4 - 5	24	21.42
3.	6 or more	3	2.68
	Total	112	

75.89% cycles were of a duration of flow of 2-3 days, 21.42% cycles showed a flow of 4-5 days. Only 2.68% cycles had 6 days or longer duration of flow.

TABLE - VII

Distribution of treatment cycles according to the amount of flow.

S.No.	Amount of flow	Number of cycle	Percentage
1.	No change	78	69.64
2.	Increased	4	3.57
3.	Decreased	30	26.78
	Total	112	

The amount of menstrual flow in treatment cycles was noted as same, increased or decreased as compared with previous pretreatment cycles.

In 69.64% cycles the amount of flow remained unchanges. In 26.78% cycles the flow was reduced as compared to pretreatment cycles. In two patients 4 cycles (3.57%) were noted to be menorrhagic. One of these patients had three consecutive menorrhagic cycles while another had only one cycle with excessive flow.

TABLE - VIIIA

Effect of Centchroman on weight
0 Vs +3 months of use

S.No.	Weight in Kg.		Z (Difference between 0 and +3 values)
	0 Value	+3 Value	
1.	48	47	+ 1
2.	41	40	+ 1
3.	50	52	- 2
4.	45	42	+ 3
5.	45	45	0
6.	35	35	0
7.	53	52	+ 1
8.	39	37	+ 2
9.	35	35	0

t = 1.43, d.f. = 8, p > 0.05

d.f. = degree of freedom

In 5 patients there was a decrease in weight at 3 months. 1 patient showed an increase in weight, whereas in 3 patients the values showed neither an increase nor a decrease. On applying test of significance the changes observed were not significant ($p > 0.05$).

TABLE - VIIIB

Effect of Centchroman on weight
0 Vs +6 months of use

S.No.	Weight in Kg.		Z Difference between 0 and +6 value
	0 Value	+6 Value	
1.	48	41	+ 7
2.	41	41	0
3.	42	41	+ 1
4.	50	49	+ 1
5.	34	35	- 1
6.	39	37	+ 2
7.	45	43	+ 3
8.	46	45	+ 1
9.	45	44	+ 1
10.	35	37	- 2
11.	45	45	0

$t = 0.70$, d.f. = 10, $p > 0.05$

When the weight changes at +6 months were compared with the initial (0) weight two cases showed an increase, two

cases showed no change while 7 cases showed a reduction in weight. These changes were again statistically insignificant.

TABLE - VIIIC

Effect of Centchroman on weight
0 Vs +12 months of use

S. No.	Weight in Kg.		Z Difference between 0 and +12 value
	0 Value	+12 value	
1.	48	41	+ 7
2.	41	41	0
3.	50	45	+ 5
4.	45	41	+ 4

t = 2.72, d.f. = 3, p $\nearrow$ 0.05

Three out of four patients showed a decrease in weight at +12 months as compared to initial weight. Fourth patient showed no change. These changes were found to be insignificant on applying test of significance.

Clinical observations :

Nothing abnormal was diagnosed on physical and per vaginum examination in the volunteers during the trial. Findings of physical examination were within normal limits.

B: BIOCHEMICAL TESTS :

Distribution of cases according to findings of urinalysis in pretreatment and treatment cycles shown in Table IXA and IXB.

TABLE - IXA

Sl. No.	URINE ALBUMIN*							
	0		+3		+6		+12	
	No.	%	No.	%	No.	%	No.	%
1. Nil	11	61.11	7	77.78	11	100.00	4	100.00
2. Traces	6	33.33	2	22.22	-	-	-	-
3. Present	1	5.56	-	-	-	-	-	-

TABLE - IXB

Sl. No.	URINE SUGAR*							
	0		+3		+6		+12	
	No.	%	No.	%	No.	%	No.	%
1. Nil	16	88.89	7	77.78	11	100.00	4	100.00
2. Traces	1	5.56	2	22.22	-	-	-	-
3. Present	1	5.56	-	-	-	-	-	-

*At +3 months only 9 cases turned up whereas at +6 months 11 patients were followed. At +12 months only 4 cases could be followed. Therefore the percentage of cases showing a

particular finding of urine examination has been calculated taking the respective number of patients that turned up.

Only one patient (5.56%) showed presence of urine albumin at 0 months. 6 cases (33.33%) showed traces of albumin whereas 61.11% cases (11 in number) showed absence of urine albumin.

At +3 months 77.78% (7 cases) showed Nil urine albumin. 22.22% cases (2 in number) showed traces of albumin.

At +6 months and +12 months all the patients showed Nil urine albumin.

One patient (5.56%) each showed urine sugar + and in traces at 0 months. At +3 months 22.22% (2 cases) showed traces of urine sugar. 77.78% cases showed absent urine sugar.

At +6 months and +12 months all the patients followed showed absence of urine sugar.

TABLE - XA

Effect of Centchroman on Hb gm%

0 Vs +3 months

S.No.	Haemoglobin gm%		Z (Difference between the two)
	0 value	+3 value	
1.	11.0	11.5	- 0.5
2.	9.5	10.0	- 0.5
3.	10.0	12.0	- 2.0
4.	10.5	10.0	+ 0.5
5.	11.5	11.0	+ 0.5
6.	10.0	10.5	- 0.5
7.	8.0	9.0	- 1.0
8.	11.0	12.0	- 1.0
9.	10.0	8.0	+ 2.0

t = 0.44, d.f. = 8, p > 0.05

Six patients showed an increase in the Hb gm% at +3 months as compared with 0 value. 3 patients showed a fall at +3 months. However these differences were statistically insignificant.

TABLE - XB

Effect of Centchroman on haemoglobin gm%

0 vs +6 months

S.No.	Haemoglobin gm%		Z (Difference between the two)
	0 value	+6 value	
1.	11.0	9.0	+ 2.0
2.	9.5	10.0	- 0.5
3.	12.0	11.8	+ 0.2
4.	10.0	10.5	- 0.5
5.	11.5	11.0	+ 0.5
6.	12.0	12.0	0
7.	10.5	11.0	- 0.5
8.	11.0	10.0	+ 1.0
9.	11.5	11.0	+ 0.5
10.	10.0	10.5	- 0.5
11.	10.0	10.4	- 0.4

t = 0.66, d.f. = 10, p > 0.05

5 patients showed a decrease and 5 patients showed an increase in the Haemoglobin levels at +6 months in comparison to initial values. One patient showed no change. Here again the differences were statistically insignificant.

TABLE - XC

Effect of Centchroman on haemoglobin gm%

0 Vs +12 months

S.No.	Haemoglobin gm%		Z (Difference between the two)
	0 value	+12 value	
1.	11.0	10.8	+ 0.2
2.	9.5	9.8	- 0.3
3.	10.0	11.0	- 1.0
4.	10.5	10.8	- 0.3

t = 1.65, d.f. = 3, p > 0.05

At +12 months 3 patients showed an increase and one patient showed decrease in haemoglobin value as compared to initial values. But the differences were found to be statistically insignificant.

TABLE - XIA

Effect of Centchroman on serum bilirubin levels

0 Vs +3 months

S.No.	Serum Bilirubin		Z (Difference between the two)
	0 value	+3 value	
1.	1.0	0.6	+ 0.4
2.	0.8	1.0	- 0.2
3.	1.0	0.7	+ 0.3
4.	1.6	1.4	+ 0.2
5.	0.8	0.6	+ 0.2
6.	1.5	1.0	+ 0.5
7.	0.7	0.6	+ 0.1
8.	1.2	1.0	+ 0.2
9.	1.0	0.8	+ 0.2

$t = 3.15$, $d.f. = 8$, $p < 0.05$

In 8 out of 9 cases a fall in serum bilirubin level was noted. One case showed increase in the value at +3 months. On applying the test of significance this fall in serum bilirubin level was found to be statistically significant.

TABLE - XIB

Effect of Centchroman on serum bilirubin levels

0 Vs +6 months

S.No.	Serum Bilirubin		Σ (Difference between the two)
	0 value	+6 value	
1.	1.0	0.6	+ 0.4
2.	0.8	0.7	+ 0.1
3.	0.6	0.8	- 0.2
4.	1.0	1.2	- 0.2
5.	1.3	1.0	+ 0.3
6.	0.5	0.7	- 0.2
7.	1.6	0.8	+ 0.8
8.	1.0	1.2	- 0.2
9.	0.8	0.6	+ 0.2
10.	1.5	1.0	+ 0.5
11.	0.7	0.6	+ 0.1

$t = 1.51$, $d.f. = 10$, $p > 0.05$

At +6 months 7 cases showed a fall in serum bilirubin whereas 4 cases showed an increase in serum bilirubin value. However this change was found to be statistically insignificant.

TABLE - XIC

Effect of Centchroman on serum bilirubin levels

0 Vs +12 months

S.No.	Serum Bilirubin		Z (Difference between the two)
	0 value	+12 value	
1.	1.0	0.7	+ 0.3
2.	0.8	0.7	+ 0.1
3.	1.0	1.0	0
4.	1.6	1.0	+ 0.6

t = 1.92, d.f. = 3, p > 0.05

At +12 months serum bilirubin levels showed a fall in 3 patients when compared with initial values. One patient showed no change. However this fall was statistically found to be insignificant.

TABLE - XIIA

Effect of Centchroman on S.G.P.T. levels

0 Vs +3 months

S.No.	S.G.P.T.		Z (Difference between the two)
	0 value	+3 value	
1.	12	10	+ 2
2.	7	9	- 2
3.	14	12	+ 2
4.	12	12	0
5.	12	10	+ 2
6.	15	13	+ 2
7.	11	10	+ 1

t = 1.72, d.f. = 6, p > 0.05

Comparison of S.G.P.T. values at +3 months and 0 months showed that 5 patients recorded a fall in S.G.P.T. values. One patient each showed no change and increase in value at 3 months. The changes were statistically insignificant.

TABLE - XXXB

Effect of Centchroman on S.G.P.T. levels
0 Vs +6 months

S.No.	S.G.P.T.		Z
	0 value	+6 value	(Difference between the two)
1.	12	10	+ 2
2.	12	10	+ 2
3.	13	10	+ 3
4.	7	13	- 6
5.	11	9	+ 2
6.	14	11	+ 3
7.	12	13	- 1
8.	10	10	0

t = 0.59, d.f. = 7, p 7 0.05

From this table it can be seen that 5 patients showed fall in S.G.P.T. levels at 3 months, 2 patients showed an increase and one patient showed no change. The changes here are again not statistically significant.

TABLE - XIIC

Effect of centchroman on S.G.P.T. levels

0 Vs +12 months

S.No.	S.G.P.T.		Z (Difference between the two)
	0 value	+12 value	
1.	12	9	+ 3
2.	7	10	- 3
3.	12	13	+ 1

t = 0.19, d.f. = 2, p > 0.05

The differences noted at +12 months were found to be statistically insignificant.

TABLE - XIIIA

Effect of Centchroman on blood urea

0 Vs +3 months

S.No.	Blood urea		Z (Difference between the two)
	0 value	+3 value	
1.	23	20	+ 3
2.	10	18	- 8
3.	40	30	+10
4.	33	33	0
5.	40	34	+ 6
6.	20	22	- 2
7.	27	26	+ 1
8.	40	34	+ 6
9.	20	24	- 4

t = 0.71, d.f. = 8, p > 0.05

At +3 months the change in blood urea levels was found to be statistically insignificant.

TABLE - XIIIB

Effect of Centchroman on Blood urea

0 Vs +6 months

S.No.	Blood Urea		Z (Difference between the two)
	0 value	+6 value	
1.	23	30	- 7
2.	10	20	- 10
3.	22	28	- 6
4.	40	46	- 6
5.	36	30	+ 6
6.	20	23	- 3
7.	33	30	+ 3
8.	20	24	- 4
9.	40	32	+ 8
10.	20	22	- 2
11.	23	20	+ 3

t = 0.93, d.f. = 10, p $\rightarrow$ 0.05

Here 7 out of 11 cases showed an increase in blood urea levels at +6 months. 4 cases showed a fall in the urea levels at +6 months. But the changes noted was found to be statistically insignificant on applying t test.

TABLE - XIIIC

Effect of Centchroman on Blood Urea

0 Vs +12 months

S.No.	Blood Urea		Z (Difference between the two)
	0 value	+12 value	
1.	23	26	- 3
2.	10	26	-16
3.	40	32	+ 8
4.	33	30	+ 3

t = 0.39, d.f. = 3, p $\rightarrow$ 0.05

Here again the changes noted were statistically insignificant.

TABLE - XIVA

Effect of Centchromen on serum creatinine levels

0 Vs +3 months

S.No.	Serum creatinine		Z (Difference between the two)
	0 value	+3 value	
1.	1.2	1.0	+ 0.2
2.	1.2	1.0	+ 0.2
3.	1.4	1.5	- 0.1
4.	1.5	1.4	+ 0.1
5.	1.5	1.5	0
6.	1.2	1.0	+ 0.2
7.	1.2	1.2	0
8.	1.5	1.3	+ 0.2
9.	1.0	1.0	0

t = 0.34, d.f. = 8, p > 0.05

5 out of 9 cases showed a fall in serum creatinine levels at +3 months, 3 cases showed no change and one case showed an increase. On applying the test of significance these changes were found to be insignificant.

TABLE - XIVB

Effect of Centchroman on serum creatinine levels

0 Vs +6 months

S.No.	Serum creatinine		Z (Difference between the two)
	0 value	+6 value	
1.	1.2	1.5	- 0.3
2.	1.2	1.0	+ 0.2
3.	1.4	1.6	- 0.2
4.	1.0	1.2	- 0.2
5.	2.5	1.0	+ 1.5
6.	1.5	1.5	0
7.	1.5	1.4	+ 0.1
8.	1.5	1.3	+ 0.2
9.	1.2	1.3	- 0.1
10.	1.5	1.3	+ 0.2

t = 0.87, d.f. = 9, p > 0.05

The difference noted in serum creatinine values at 0 and +6 months of drug use were statistically insignificant.

TABLE - XIVC

Effect of Centchroman on serum creatinine levels

0 Vs +12 months

S.No.	Serum creatinine		Z (Difference between thetwo)
	0 value	+12 value	
1.	1.2	1.2	0
2.	1.2	1.2	0
3.	1.4	1.2	+ 0.2
4.	1.5	1.2	+ 0.3

t = 1.86 d.f. = 3, p > 0.05

Out of 4 patients 2 showed no change in serum creatinine value at +12 months compared to 0 value. The other two patients showed a fall in serum creatinine level at +12 months when compared with 0 value. Application of test of significance showed this fall to be insignificant statistically.

TABLE - XVA

Effect of Centchromen on Serum Cholesterol

0 Vs +3 months

S.No.	Serum Cholesterol		Σ (Difference between the two)
	0 value	+3 value	
1.	175	170	+ 5
2.	140	146	- 6
3.	150	158	- 8
4.	208	200	+ 8
5.	130	135	- 5
6.	235	240	- 5
7.	180	184	- 4
8.	150	146	+ 4
9.	150	158	- 8

t = 1.04, d.f. = 8, p > 0.05

The observed changes in serum cholesterol levels at +3 months as compared to 0 levels were found to be statistically insignificant.

TABLE - XV B

Effect of Centchroman on serum cholesterol
0 Vs +6 months

S.No.	Serum cholesterol		Z (Difference between the two)
	0 Value	+6 Value	
1.	175	176	- 1
2.	140	142	- 2
3.	190	185	+ 5
4.	150	158	- 8
5.	185	188	- 3
6.	165	168	- 3
7.	208	200	+ 8
8.	200	190	+10
9.	130	138	- 8
10.	235	240	- 5
11.	145	140	- 5

t = 0.24, d.f. = 10, p > 0.05

At +6 months 7 out of 11 patients showed an increase in serum cholesterol levels. 4 patients showed a fall in the serum cholesterol levels at 6 months. But the observations were found to be statistically insignificant.

TABLE - XVC

Effect of Centchroman on serum cholesterol
0 Vs +12 months

S.No.	Serum cholesterol		Z (Difference between the two)
	0 Value	+12 Value	
1.	175	165	+ 10
2.	140	155	- 15
3.	150	145	+ 5
4.	208	190	+ 18

t = 0.94, d.f. = 3, p > 0.05

Differences noted in serum cholesterol levels at +12 months and 0 month were found to be statistically insignificant.

TABLE - XVIA

Effect of Centchroman on serum Triglycerides
0 Vs +3 months

S.No.	Serum Triglyceride		Z (Difference between the two)
	0 Value	+3 Value	
1.	80	78	+ 2
2.	95	98	- 3
3.	95	90	+ 5
4.	56	60	- 4
5.	66	70	- 4
6.	110	114	- 4
7.	85	80	+ 5
8.	100	105	- 5
9.	88	96	- 8

t = 1.35, d.f. = 8, p > 0.05

Observed changes in serum triglyceride levels at +3 months were found to be statistically insignificant.

TABLE - XVIB

Effect of Centchroman on serum triglycerides
0 Vs +6 months

S.No.	Serum triglyceride		Z (Difference between the two)
	0 Value	+6 Value	
1.	80	78	+ 2
2.	95	92	+ 3
3.	71	74	- 3
4.	95	96	- 1
5.	84	78	+ 6
6.	120	122	- 2
7.	56	58	- 2
8.	95	100	- 5
9.	66	72	- 6
10.	110	110	0
11.	95	98	- 3

t = 0.93, d.f. = 10, p > 0.05

7 out of 11 cases showed an increase in serum triglyceride value at +6 months. 3 cases showed a fall in values at +6 months. 1 case showed no change in value.

The test of significance when applied showed this change to be insignificant.

TABLE - XVIC

Effect of Centchroman on serum triglycerides
0 Vs +12 months

S.No.	Serum triglyceride		Z (Difference between the two)
	0 Value	+12 Value	
1.	80	80	0
2.	95	98	- 3
3.	95	100	- 5
4.	56	66	- 10

t = 2.14, d.f. = 3, p 7 0.05

The increase in serum triglyceride at +12 months as compared to initial values was statistically found not to be significant.

C: SPECIAL TESTS :

I. Ovarian size - Ultrasonographic measurement of ovarian size was done at 0, +3, +6 and +12 months of drug use.

Ovarian size was expressed in terms of ovarian volume using the ellipsoid formula.

Ovarian volume = L x A P x T x 0.5 cc.

WHERE : L = Longitudinal axis.

A P = Anteroposterior axis

T = Transverse axis.

TABLE - XVIIA

Effect of Centchroman on ovarian size
0 Vs +3 months

S.No.	Ovarian volume (C.C.)		Z (Difference between the two)
	0 value	+ 3 value	
1.	2.97	88.50	- 85.53
2.	3.18	19.89	- 16.71
3.	4.72	5.89	- 1.17
4.	3.96	9.04	- 5.08
5.	4.17	4.59	- 0.42
6.	5.10	4.21	+ 0.89
7.	4.39	16.38	- 11.99
8.	5.19	16.67	- 11.48
9.	7.49	7.76	- 0.27

t = 1.61, d.f. = 8, p > 0.05

It is clearly evident from the above table that there is an increase of ovarian size at +3 months as compared with initial values. There is a variable rise in 8 patients and one patient showed decrease in ovarian size. The rise is however statistically not significant.

TABLE - XVIIB

Effect of Centchroman on ovarian size

0 Vs +6 months

S.No.	Ovarian volume(c.c.)		Z (Difference between the two)
	0 value	+6 value	
1.	2.97	14.18	- 11.21
2.	3.18	12.28	- 9.10
3.	4.05	13.02	- 8.97
4.	4.72	5.78	- 1.06
5.	2.92	14.18	- 11.26
6.	4.02	7.35	- 3.33
7.	3.96	22.95	- 18.99
8.	7.26	7.49	- 0.23
9.	4.17	9.99	- 5.82
10.	5.10	5.29	- 0.19

t = 3.67, d.f. = 9, $p < 0.01$

Ovarian volume at +6 months when compared with initial level was found to increase in all the cases. This increase in ovarian volume was found to be statistically significant ($p < 0.01$).

VARIATION OF OVARIAN SIZE

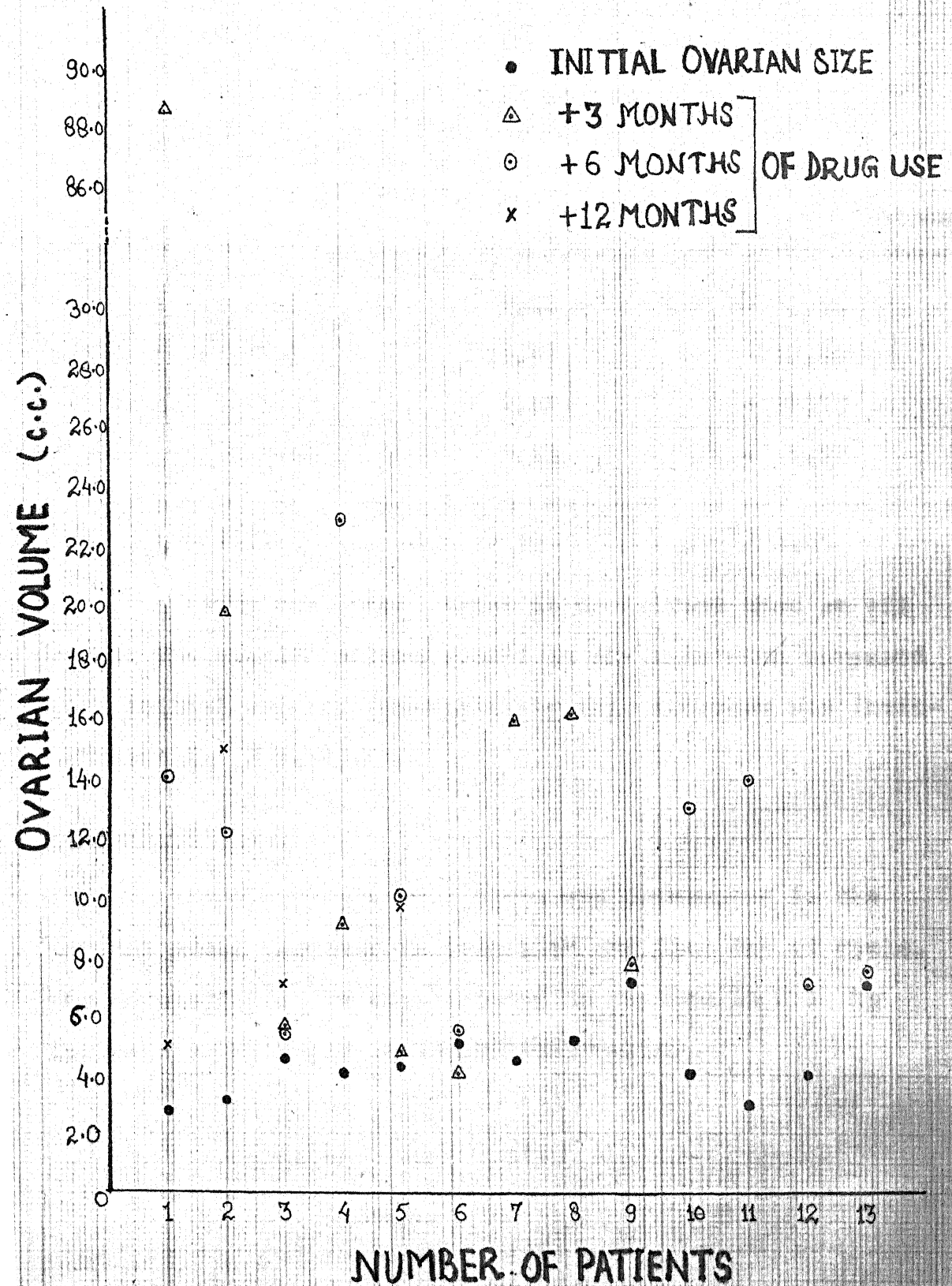


TABLE - XVIIC

Effect of Centchroman on ovarian size
0 Vs +12 months

S.No.	Ovarian volume(c.c.)		Z (Difference between the two)
	0 value	+12 value	
1.	2.97	5.28	- 2.31
2.	3.18	15.62	- 12.44
3.	4.72	7.49	- 2.77
4.	3.96	9.13	- 5.17

t = 2.42, d.f. = 3, p > 0.05

From the above table it is evident that at +12 months the ovarian volume showed an increase when compared with initial values. Statistically this increase was insignificant (p > 0.05).

Vaginal Cytology :

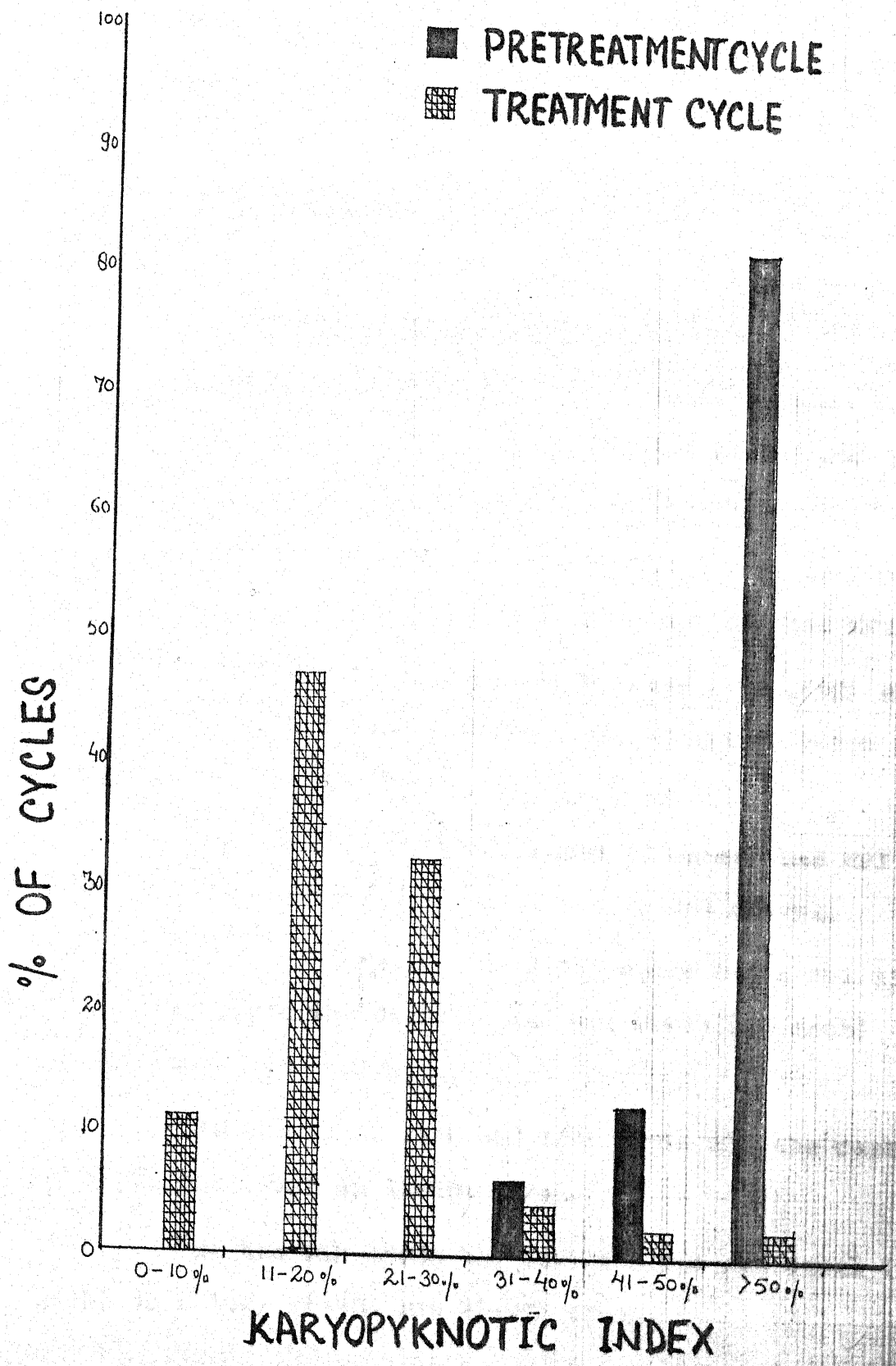
The karyopyknotic index was determined in the varinal smear prepared on 14th/15th and 21st day of cycle. The maturation index corresponded to changes in KPI by increase or decrease in superficial cells.

TABLE - XVIIIA

Distribution of cases according to karyopykmotic index on 14th/15th day of cycle in the pretreatment cycle (Initial smear) and various treatment cycles.

Sl. No.	Cycle No.	No. of Pt. in follow up	0-10% No.	%	11-20% No.	%	21-30% No.	%	31-40% No.	%	41-50% No.	%	7 50% No.	%
1.	0	17	-	-	-	-	-	-	1	5.88	2	11.76	14	82.35
2.	1	11	-	-	2	18.19	4	36.36	2	18.19	1	9.09	2	18.19
3.	2	11	-	-	3	27.27	7	63.64	-	-	1	9.09	-	-
4.	3	9	1	11.11	4	44.44	3	33.33	1	11.11	-	-	-	-
5.	4	8	1	12.50	4	50.00	3	37.50	-	-	-	-	-	-
6.	5	9	3	33.33	4	44.44	2	22.22	-	-	-	-	-	-
7.	6	8	2	25.00	4	50.00	2	25.00	-	-	-	-	-	-
8.	7	5	-	-	5	100.00	-	-	-	-	-	-	-	-
9.	8	4	-	-	2	50.00	2	50.00	-	-	-	-	-	-
10.	9	3	-	-	3	100.00	-	-	-	-	-	-	-	-
11.	10	3	1	33.33	1	33.33	1	33.33	-	-	-	-	-	-
12.	11	1	-	-	1	100.00	-	-	-	-	-	-	-	-
13.	12	2	-	-	2	100.00	-	-	-	-	-	-	-	-

In the pretreatment cycle 14 cases (82.35%) showed karyopykmotic index more than 50% and 2 cases (11.76%) had karyopykmotic index between 41-50%. One case (5.88%) had KPI between 31-40%. In this particular case the smear showed presence of trichomonas vaginalis.


KARYOPYKNOTIC INDEX ON 14TH DAY OF CYCLE
PRETREATMENT CYCLE
TREATMENT CYCLE
% OF CYCLES
100
90
80
70
60
50
40
30
20
10
0
0-10%
11-20%
21-30%
31-40%
41-50%
>50%
KARYOPYKNOTIC INDEX

In the first treatment cycle 4 cases (36.36%) had KPI between 21-30%. 2 cases (18.19%) each had KPI in 10-20%, 31-40% and 7 50% groups. 1 case (9.09%) had KPI 41-50%.

In the second treatment cycle, 7 cases (63.64%) had KPI 21-30%. 3 cases (27.27%) had KPI 11-20% and 1 case (9.09%) had KPI 41-50%. No case showed KPI 7 50%.

In the third cycle out of 9 cases 4 cases (44.44%) had KPI 11-20%. 3 cases (33.33%) had KPI 21-30% and 11.11% cases (1 case) each had KPI 0-10% and 31-40%.

In the 4th cycle 50% cases had KPI 11-20% and three cases (37.50%) had KPI 21-30%. 1 case (12.50%) had KPI 0-10%.

In the 5th cycle out of 9 cases 4 (44.44%) showed KPI 11-20% while 3 cases (33.33%) showed KPI of 0-10% 2 cases (22.22%) had KPI 21-30%.

In the 6th cycle 50% cases (4 cases) had KPI 11-20% and 25% cases each were in 0-10% and 21-30% groups.

All the 5 cases in the 7th cycle had a KPI 11-20%. In the 8th cycle the distribution was equal(50% each) in 11-20% and 21-30% group.

In the 10th, 11th and 12th cycle all the cases followed had a KPI in 11-20% group.

In the 9th cycle the distribution was equal (1 case each) in 0-10%, 11-20% and 21-30% group.

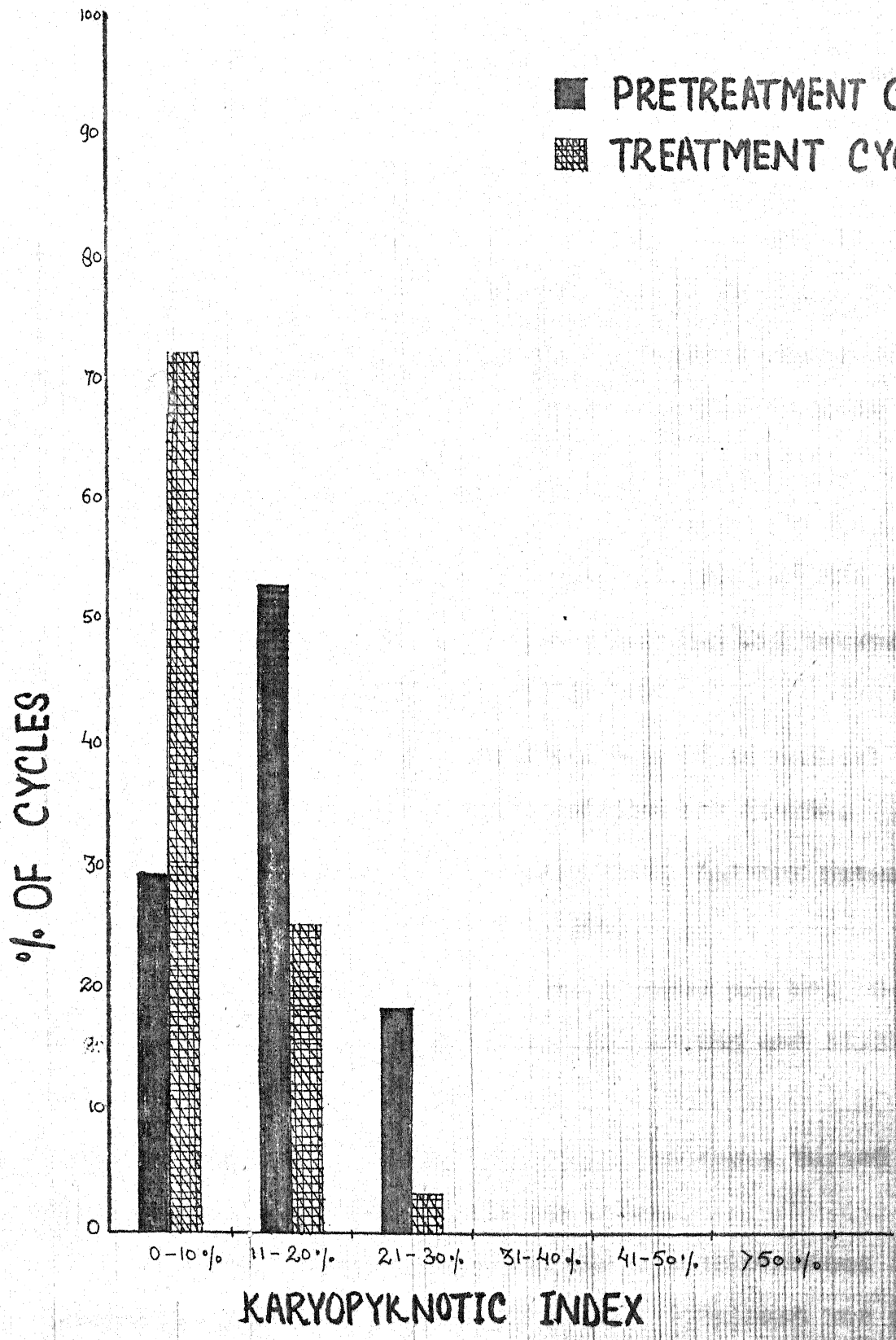
TABLE - XVIIIB

Distribution of cases according to karyopyknotic index on 21st day of cycle in the pretreatment cycle and various treatment cycles.

S.No.	Cycle	No.of Pt.in follow up	0-10%		11-20%		21-30%	
			No.	%	No.	%	No.	%
1.	0	17	5	29.41	9	52.94	3	17.65
2.	1	12	6	50.00	5	41.67	1	8.33
3.	2	9	6	66.67	3	33.33	-	-
4.	3	7	4	57.14	2	28.57	1	14.29
5.	4	9	7	77.78	2	22.22	-	-
6.	5	9	7	77.78	2	22.22	-	-
7.	6	7	6	85.71	1	14.29	-	-
8.	7	8	7	87.50	1	12.50	-	-
9.	8	4	3	75.00	1	25.00	-	-
10.	9	3	3	100.00	-	-	-	-
11.	10	3	1	33.33	2	66.67	-	-
12.	11	2	2	100.00	-	-	-	-
13.	12	2	2	100.00	-	- -	-	-

In the pretreatment cycle 9 out of 17 cases (52.94%) had KPI between 11-20%. 5 cases (29.41%) had KPI 0-10% and 3 cases (17.65%) had a KPI between 21-30%.

KARYOPYKNOTIC INDEX ON 21ST DAY OF CYCLE



In the first treatment cycle 50% cases (6 in number) had KPI between 0-10%. 41.67% (5 cases) had KPI 11-20% while one case (8.33%) had KPI 21-30%.

In the 2nd treatment cycle 6 cases (66.67%) had KPI 0-10% and 3 cases (33.33%) had KPI 11-20%.

In the 3rd cycle 57.14% (4 cases) had KPI 0-10% and 2 cases (28.57%) had a KPI between 11-20%. 1 case comprising 14.29% had KPI 21-30%.

In the 4th and 5th cycle 7 cases comprising a total of 77.78% had KPI 0-10% and 2 cases (22.22%) had KPI 11-20%.

In the 6th cycle 85.71% cases had KPI between 0-10% and 1 case (14.29%) had KPI 11-20%.

In the 7th cycle 87.50% cases (7 in number) had KPI 0-10% whereas 1 case (12.50%) showed KPI 11-20%.

In the 8th cycle 3 cases (75%) had KPI between 0-10% and 1 case (25%) had KPI 11-20%.

In the 9th cycle all the 3 cases had KPI 0-10%. In the 10th cycle 66.67% cases had KPI 11-20% and 33.33% cases had KPI of 0-10%.

In the 11th and 12th cycle two cases turned up for follow up and both had KPI between 0-10%.

Vaginal smear was positive for trichomonas in pre-treatment cycle in 2 patients for which treatment was given. In no other cycle presence of trichomonas or monilia could be demonstrated.

III. CERVICAL MUCUS TEST :

(i) Spinbarkeit test

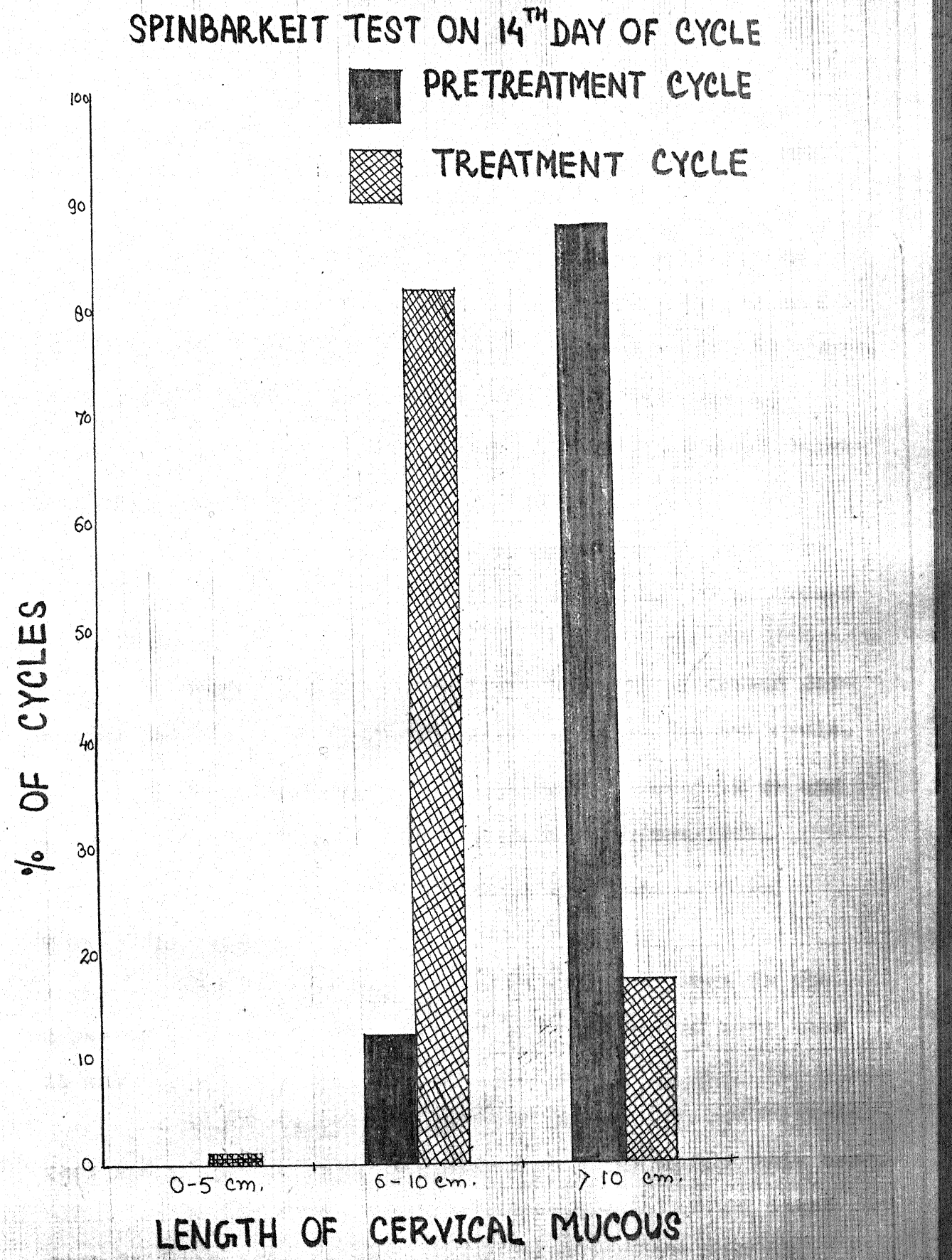
(ii) Fern test.

TABLE - XIX

Distribution of cases according to Spinbarkeit test on 14th / 15th day of pretreatment and treatment cycles.

S.No.	Cycle	No.of Pt.in follow up	0-5 cm		6-10 cm		7 10 cm	
			No.	%	No.	%	No.	%
1.	0	17	-	-	2	11.76	15	88.24
2.	1	11	-	-	9	81.82	2	18.18
3.	2	11	1	9.09	8	72.73	2	18.18
4.	3	9	-	-	7	77.78	2	22.22
5.	4	8	-	-	7	87.50	1	12.50
6.	5	9	-	-	8	88.89	1	11.11
7.	6	7	-	-	5	71.43	2	28.57
8.	7	4	-	-	4	100.00	-	-
9.	8	4	-	-	3	75.00	1	25.00
10.	9	3	-	-	2	66.67	1	33.33
11.	10	3	-	-	3	100.00	-	-
12.	11	1	-	-	1	100.00	-	-
13.	12	2	-	-	2	100.00	-	-

In the pretreatment cycle 15 out of 17 cases (88.24%) showed spinbarkeit test of more than 10 cm and 2 cases (11.76%) showed spinbarkeit test between 6-10 cm.


SPINBARKEIT TEST ON 14TH DAY OF CYCLE
PRETREATMENT CYCLE
TREATMENT CYCLE
100
90
80
70
60
50
40
30
20
10
0
% OF CYCLES
0-5 cm.
6-10 cm.
> 10 cm.
LENGTH OF CERVICAL MUCOUS

In the first treatment cycle 9 out of 11 cases (81.82%) showed spinbarkeit of 6-10 cm and 2 cases (18.18%) showed more than 10 cm spinbarkeit test.

In the 2nd cycle 72.73% cases (8 in number) had 6-10 cm spinbarkeit formation whereas 2 cases (18.18%) had 10 cm spinbarkeit test. 1 case (9.09%) was in 0-5 cm range.

In the 3rd cycle 77.78% cases (7) had 6-10 cm spinnability whereas 22.22% (2 cases) showed a thread formation of more than 10 cm.

In the 4th cycle 87.50% cases (7 cases) were in 6-10 cm range and 12.50% (1 case) had more than 10 cm thread formation.

88.89% cases (8 in number) had 6-10 cm thread formation and 11.11% (1 case) more than 10 cm in the 5th cycle.

In the 6th cycle 5 cases (71.43%) had 6-10 cm and 2 cases (28.57%) had more than 10 cm thread formation.

In the 7th cycle all the 4 cases had 6-10 cm thread formation.

In the 8th cycle 75% cases (3 cases) were in the range of 6-10 cm and 25% (1 case) in the range of more than 10 cm.

66.67% cases (2 in number) had 6-10 cm spinbarkeit test and 33.33% (1 case) had more than 10 cm spinbarkeit test.

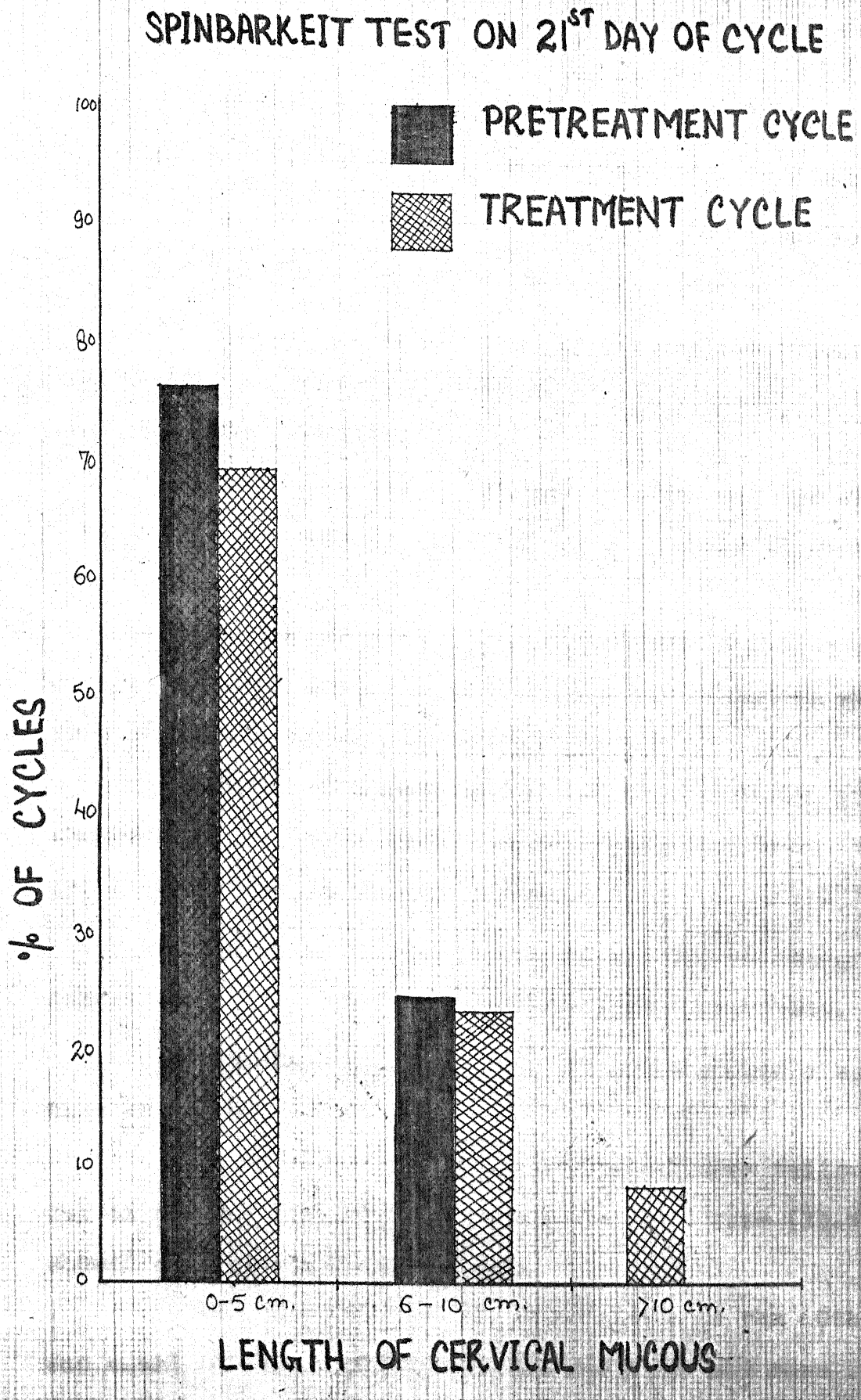
In the 10th, 11th and 12th cycle 3,1 and 2 cases were followed and all had a spinbarkeit test of 6-10 cm.

TABLE - XX

Distribution of cases according to spinbarkeit test on 21st day of pretreatment and treatment cycle.

S.No.	Cycle	No.of pt.in follow up	0 - 5cm No.	%	6-10 cm No.	%	7 10 cm No.	%
1.	0	17	13	76.47	4	23.53	-	-
2.	1	12	8	66.67	4	33.33	-	-
3.	2	8	4	50.00	4	50.00	-	-
4.	3	7	5	71.43	-	-	2	28.57
5.	4	9	7	77.78	1	11.11	1	11.11
6.	5	9	6	66.67	2	22.22	1	11.11
7.	6	7	4	57.14	2	28.57	1	14.29
8.	7	8	6	75.00	2	25.00	-	-
9.	8	4	4	100.00	-	-	-	-
10.	9	3	2	66.67	1	33.33	-	-
11.	10	3	1	33.33	1	33.33	1	33.33
12.	11	2	2	100.00	-	-	-	-
13.	12	2	2	100.00	-	-	-	-

In the pretreatment cycle 76.47% cases (13 cases) showed spinbarkeit test of 0-5 cm and 23.53% (4 cases) showed 6-10 cm test.


SPINBARKEIT TEST ON 21ST DAY OF CYCLE
PRETREATMENT CYCLE
TREATMENT CYCLE
100
90
80
70
60
50
40
30
20
10
0
% OF CYCLES
0-5 cm.
6-10 cm.
>10 cm.
LENGTH OF CERVICAL MUCOUS

In the first treatment cycle 66.67% cases showed spinbarkeit of 0-5 cm and 33.33% showed 6-10 cm spinbarkeit.

In the 2nd cycle the distribution of the cases was equal (50% each) in 0-5 cm and 6-10 cm. group.

In the 3rd cycle 5 out of 7 cases comprising 71.43% showed 0-5 cm spinbarkeit whereas 2 cases (28.57%) showed more than 10 cm spinbarkeit.

In the 4th cycle 7 cases (77.78%) had 0-5 cm and 1 case (11.11%) each had 6-10 cm and more than 10 cm thread formation.

In the 5th cycle 6 cases (66.67%) showed 0-5 cm, 2 cases (22.22%) showed 6-10 cm and 1 case (11.11%) showed more than 10 cm thread formation.

57.14% (4 cases) showed 0-5 cm and 28.57% (2 cases) showed 6-10 cm and 14.29% (1 case) showed more than 10 cm spinbarkeit test in the 6th cycle.

75% cases in the 7th cycle had 0-5 cm thread formation and 25% cases had 6-10 cm thread formation.

All the 4 cases in the 8th cycle showed a spinbarkeit test of 0-5 cm.

In the 9th cycle only 3 patients were followed. Out of these 2 (66.67%) showed 0-5 cm and 1 case (33.33%) showed 6-10 cm spinbarkeit test.

The distribution of the 3 cases in the 10th cycle was equal (1 case each) in 0-5 cm, 6-10 cm, and more than 10 cm group.

Only 2 cases could be followed in the 11th and 12 th cycle and both had 0-5 cm spinbarkeit test.

TABLE - XXI

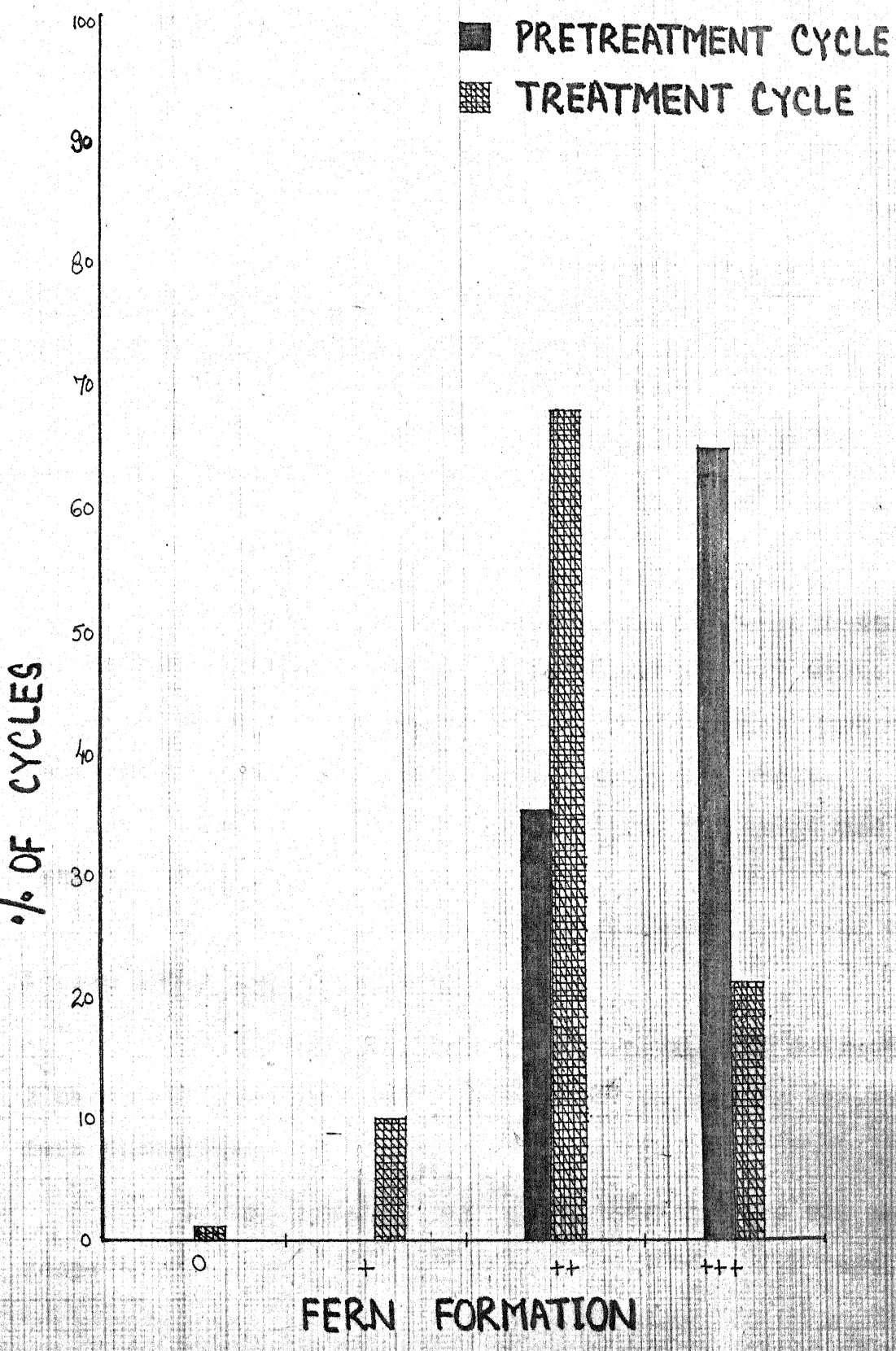
Distribution of cases according to fern test on 14th/15th day of pretreatment and treatment cycles.

S.No.	Cycle	No.of Pt.in follow up	0		+		++		+++	
			No.	%	No	%	No	%	No	%
1.	0	17	-	-	-	-	6	35.29	11	64.71
2.	1	11	-	-	1	9.09	6	54.55	4	36.36
3.	2	11	1	9.09	-	-	9	81.82	1	9.09
4.	3	9	-	-	2	22.22	6	66.67	1	11.11
5.	4	8	-	-	-	-	7	87.50	1	12.50
6.	5	7	-	-	2	28.57	2	28.57	3	42.86
7.	6	8	-	-	2	25.00	4	50.00	2	25.00
8.	7	5	-	-	-	-	5	100.00	-	-
9.	8	4	-	-	-	-	3	75.00	1	25.00
10.	9	3	-	-	-	-	2	66.67	1	33.33
11.	10	3	-	-	-	-	2	66.67	1	33.33
12.	11	1	-	-	-	-	1	100.00	-	-
13.	12	2	-	-	-	-	2	100.00	-	-

Ferning reaction was graded as recommended by Macdonald.

0 - No fern formation
\+ - Slightly positive (Weak)
++ - Moderately positive
+++ - Strongly positive

FERN TEST ON 14TH DAY OF CYCLE



In the pretreatment cycle 11 cases (64.71%) showed +++ fern test and 6 cases (35.29%) showed ++ fern test.

In the first treatment cycle 6 cases (54.55%) showed ++, 4 cases (36.36%) +++ and 1 case (9.09%) + fern test.

In the 2nd cycle 81.82% (9 cases) showed ++, 1 case each (9.09%) showed 0 and +++ fern formation.

In the 3rd cycle 6 cases (66.67%) had ++, 2 cases (22.22%) had + and 1 case (11.11%) had +++ fern test.

7 out of 8 cases in the 4th cycle had ++ fern test and 1 case (12.50%) had +++ ferning.

In the 5th cycle 42.86% (3 cases had +++ fern test and 28.57% (2 cases) cases each had + and ++ fern test.

In the 6th cycle 50% cases (4 in number) had ++ fern test and 2 cases each (25%) had +++ and + fern test.

All the 5 cases followed in the 7th cycle had ++ fern formation.

In the 8th treatment cycle 3 cases (75%) had ++ and 1 case (25%) had +++ fern formation.

In the 9th and 10th cycle 3 cases were followed and 2 cases (66.67%) were found to have ++ and 1 case (33.33%) +++ fern formation.

In the 11th and 12th cycle only one and two patients respectively were followed and they showed ++ fern test.

TABLE - XXXI

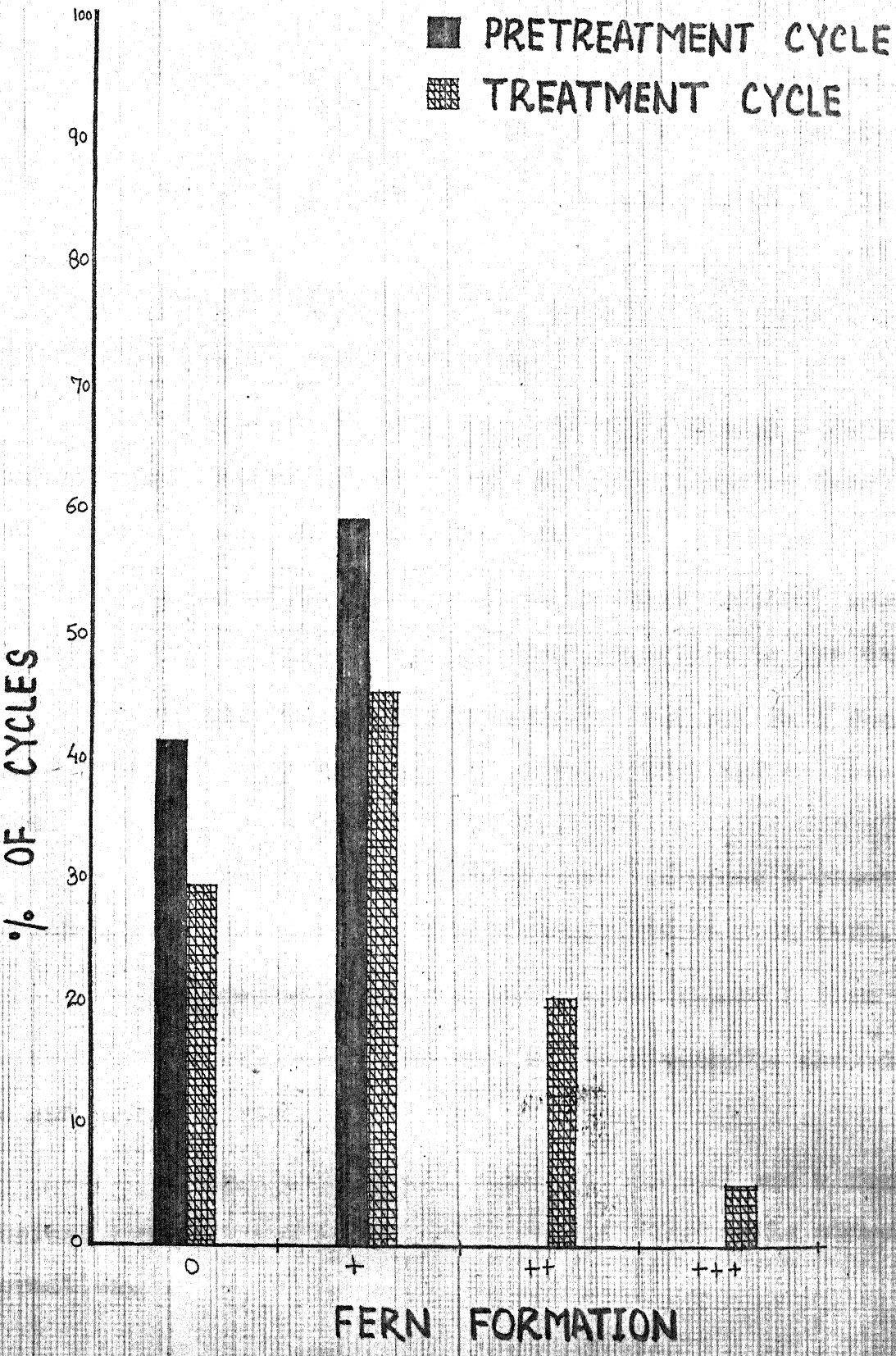
Distribution of cases according to fern test on 21st day of pretreatment and treatment cycles.

S.No.	Cycle	No.of Pt.in follow up	0		+		++		+++	
			No.	%	No.	%	No.	%	No.	%
1.	0	17	7	41.18	10	58.82	-	-	-	-
2.	1	12	4	33.33	5	41.67	3	25.00	-	-
3.	2	9	3	33.33	3	33.33	3	33.33	-	-
4.	3	7	1	14.29	4	57.14	1	14.29	1	14.29
5.	4	9	1	11.11	6	66.67	1	11.11	1	11.11
6.	5	9	2	22.11	4	44.44	2	22.22	1	11.11
7.	6	7	1	14.29	4	57.16	2	28.58	-	-
8.	7	8	4	50.00	3	37.50	1	12.50	-	-
9.	8	4	2	50.00	1	25.00	1	25.00	-	-
10.	9	3	3	100.00	-	-	-	-	-	-
11.	10	3	-	-	1	33.33	1	33.33	1	33.33
12.	11	2	1	50.00	1	50.00	-	-	-	-
13.	12	2	-	-	2	100.00	-	-	-	-

In the pretreatment cycle 10 out of 17 cases comprising 58.82% showed + and 7 cases (41.18%) showed 0 fern test.

In the first treatment cycle 41.67% (5 cases) showed +, 33.33% (4 cases) showed Nil and 25.00% (3 cases) showed ++ fern formation.

FERN TEST ON 21ST DAY OF CYCLE



In the 2nd treatment cycle 9 cases followed were distributed equally (33.33%) 3 cases in 0, + and +++ group.

In the 3rd cycle 57.14% (4 cases) showed + fern test while 1 case each (14.29%) showed, 0, ++ and +++ fern test.

In the 4th cycle 6 cases (66.67%) had + and 1 case (11.11%) each in 0, ++ and +++ group.

In the 5th cycle 4 cases (44.44%) showed + fern formation whereas 2 cases (22.22%) each showed Nil and ++ fern test and 1 case (11.11%) had +++ fern test.

4 cases (57.16%) showed +, 2 cases (28.58%) showed ++ and 1 case (14.29%) showed Nil fern formation in the 6th cycle.

In the 7th cycle 50% cases (4) had Nil fern formation and 3 cases (37.50%) had + and 1 case (12.50%) had ++ fern formation.

In the 8th cycle 4 cases were followed, 2 cases (50%) showed 0 fern test while 1 case showed + and ++ fern test.

In the 9th cycle all the 3 cases showed 0 fern test.

One case each (33.33%) in the 10th cycle showed +, ++ and +++ fern test.

In the 11th cycle 1 case each had Nil and + fern test whereas both the cases followed in the 12th cycle showed + fern formation.

TABLE - XXIIIA

Distribution of treatment cycles according to change in KPI at 21st days as compared to KPI at 14 days.

Total cycle studied	Decrease in KPI		Increase in KPI	
	No.of cycle	%	No.of cycle	%
68	59	86.76	9	13.24

In 9 out of 68 cycles there was an increase in KPI at 21 days.

TABLE - XXIIIB

Distribution of cases with increased KPI at 21 days in relation to cycle length.

Sl. No.	Cycle showing KPI*	Initial cycle duration (days)	Present cycle duration (days)	Prolonged cycle duration		Normal cycle duration or with insignificant change	
				No.	%	No.	%
1.	1^{8}	25-30	42	-	-	-	-
2.	1^{10}	25-30	31	-	-	-	-
3.	4^{10}	30	38	-	-	-	-
4.	5^{5}	27-29	36	6	66.67	3	33.33
5.	6^{5}	25	32	-	-	-	-
6.	7^{3}	25-26	42	-	-	-	-
7.	7^{6}	25-26	29	-	-	-	-
8-	14^{3}	30	36	-	-	-	-
9.	14^{4}	30	19	-	-	-	-

* The figures represent the patient number and the particular cycle in which the change is observed e.g. $7^{\cdot 3}$ means 3rd treatment cycle of patient number 7.

Further analysis showed that out of the 9 cycles showing increase in KPI at 21 days 6 cycles were prolonged when compared with initial cycle duration. Rest of the 3 cycles showed a cycle duration that was reduced or near normal.

TABLE - XXIVA

Distribution of treatment cycles according to change in Spinbarkeit at 21 days as compared to Spinbarkeit at 14 days.

Total cycle studied	Decrease in SB test value		Increase in SB test value		No change	
	No.of cycle	%	No.of cycle	%	No.of cycle	%
68	52	76.47	9	13.24	7	10.29

In 9 out of 68 cycles an increase in the value of the Spinbarkeit test was noted. In 7 cases (10.29%) no change was observed at 21 days as compared to 14 days.

TABLE - XXIVB

Distribution of cases with increased value of Spinbarkeit test at 21 days in treatment cycles in relation to cycle length.

Sl. No.	Cycle showing SB Value	Initial cycle duration (days)	Present cycle duration (days)	Prolonged cycle duration		Cycle duration normal or with insignificant chance	
				No.	%	No.	%
1.	4^{10}	30	38	-	-	-	-
2.	5^{1}	27-29	33	-	-	-	-
3.	5^{5}	27-29	36	-	-	-	-
4.	6^{5}	25	32	7	77.78	2	22.22
5.	7^{3}	25-26	42	-	-	-	-
6.	7^{6}	25-26	29	-	-	-	-
7.	14^{2}	30	36	-	-	-	-
8.	14^{3}	30	36	-	-	-	-
9.	14^{4}	30	19	-	-	-	-

Further analysis of the 9 cycles with increased Spinbarkeit value at 21 days, showed that 7 of these cycles (77.78%) were prolonged when compared with 0 cycle while 2 cycle (22.22%) showed a cycle duration that was normal or reduced.

TABLE - XXVA

Duration of treatment cycles according to change in fern test at 21 days as compared to fern test at 14 days.

Total cycles studied	Decrease in ferning		Increasing in ferning	
	No.of cycles	%	No.of cycles	%
67	58	86.57	9	13.43

Here again 9 out of a total of 67 cycles showed an increase in ferning at 21 days.

TABLE - XXVB

Distribution of cases with increased ferning at 21 days in relation to cycle length.

Sl. No.	Cycle showing SB Value	Initial cycle duration (days)	Present cycle duration (days)	Prolonged cycle duration		Cycle duration normal or with insignificant change	
				No.	%	No.	%
1.	$4^{1}10^{1}$	30	38	-	-	-	-
2.	$5^{1}1^{1}$	27-29	33	-	-	-	-
3.	$5^{1}5^{1}$	27-29	36	7	77.78	2	22.22
4.	$6^{1}5^{1}$	25	32	-	-	-	-
5.	$7^{1}3^{1}$	25-26	42	-	-	-	-
6.	$7^{1}6^{1}$	25-26	29	-	-	-	-
7.	$14^{1}2^{1}$	30	36	-	-	-	-
8.	$14^{1}3^{1}$	30	36	-	-	-	-
9.	$14^{1}4^{1}$	30	19	-	-	-	-

Out of 9 cycles showing increased ferning at 21st day as compared to 14th day. 7 cycle (77.78%) had prolonged cycle duration whereas 2 cycles showed a normal or near normal cycle duration.

TABLE - XXVI

Distribution of cases according to side effects noted treatment cycles.

S.No.	Side effect	No.of cases	Percentage
1.	Prolonged cycle	5	27.78
2.	Scanty menses	5	27.78
3.	Menorrhagia	2	11.11
4.	Reduced appetite	3	16.67
5.	Short cycle	1	5.56

Five cases (27.78%) showed prolonged cycle and the same number of cases showed scanty menses. Three cases (16.67%) showed reduced appetite. 2 cases (11.11%) showed menorrhagic cycles; one case had three consecutive menorrhagic cycles whereas the other one had only one menorrhagic cycle. Only one case reported a short cycle of 19 days duration (5.56%).

D I S C U S S I O N

DISCUSSION

The present study was undertaken on 18 healthy female volunteers of reproductive age group (20-35 years) opting for centchroman as oral contraceptive. 38.89% of these cases were in 23-25 years age group and 27.77% cases were in 26-28 years age group. 38.89% of these cases were of second parity. All the volunteers had a cycle duration of 25-35 days in the pretreatment cycle.

A total of 112 cycles of use were covered and 82.14% cycles showed 25-35 days cycle duration, 10.71% of treatment cycles were having 36-45 days cycle duration. 4.46% cycles were prolonged for more than 45 days. Rama Vaidya et al (1977) noted an increase in cycle length of all the cases in the series. This was attributed to prolongation of the follicular phase of the treatment cycle. The pattern of prolonged cycle was random and was not restricted to any particular cycle or subject. One case (5.56%) had short cycle of 19 days duration. The short and prolonged cycle did not present any consistent pattern and were not found to be restricted to individual (Annual Report C.D.R.I. 1981).

In the initial ('0') cycle 44.44% cases each had a duration of flow of 2-3 days and 4-5 days. A total of 112 treatment cycles were observed and 75.89% cycles were found to have 2-3 days duration of flow. 2.68% cycles were found to have duration of flow 6 or more days. The duration of flow in various treatment cycles did not present any consistent pattern and was not restricted to any particular volunteer.

69.64% treatment cycles showed no change in the amount of flow. In 26.78% cycles showed reduced amount of flow and 3.57% cycles showed increased flow. The pattern of excessive or scanty flow was again not restricted to any particular cycle or volunteer. V.P. Kamboj et al(1977) found in their human studies with centchroman that the disturbances of menstrual cycle had almost equal distribution in all the groups and therefore they found it difficult to assign this effect to centchroman.

In our study we did not find any significant change in pulse or blood pressure which is consistent with the findings of Dhawan et al (1977). Nothing abnormal was found on per vaginum findings.

The changes in body weight at +3 months, +6 months and +12 months when compared with initial values were found

to be statistically insignificant. This finding is in accordance with the results of the extended Phase III Clinical Trial of Centchroman carried out by C.D.R.I., Lucknow.

Biochemical Tests :

The findings of urinalysis were within normal limits in the pretreatment cycle and at +3, +6 and +12 months of use.

At +3 months 6 out of 9 patients showed an increase in haemoglobin gm% level. Similarly 50% patients showed an increase and 50% a decrease in haemoglobin gm% levels at +6 months. At +12 months 75% cases showed an increase of haemoglobin gm%. However statistically these changes were found to be insignificant.

Similarly variation in SGPT levels, blood urea and serum creatinine at +3, +6 and +12 months of drug therapy were found to be statistically insignificant.

S.N. Roy et al (1977) in their study on effect of centchroman in normospermic and oligospermic individuals found no alteration in liver and kidney function tests during drug therapy.

Das et al (1977) reported that physical and laboratory tests did not reveal any change following medication with centchroman.

Serum Bilirubin :

In our study a decrease in serum bilirubin levels at +3 months as compared with '0' value was found in 8 (88.89%) out of 9 cases. This difference was found to be statistically significant ($p < 0.05$).

At +6 months 7 cases (63.64%) showed a fall in serum bilirubin level. 4 cases showed an increase in serum bilirubin value.

Similarly at +12 months a fall in serum bilirubin was noted in 3 out of 4 cases.

On applying the test of significance the changes observed at +6 and +12 months were found to be statistically insignificant ($p > 0.05$).

Lipids :

Serum cholesterol values at +3 months were compared with initial values. 3 out of 9 patients were found to have a decrease and the rest 6 patients showed an increase. However on applying the test of significance it was found to be statistically insignificant.

Similarly at +6 months 7 out of 11 cases had an increase in serum cholesterol value. But this observed difference was found to be insignificant.

No statistically significant difference was noted at +12 months when compared with 0 values.

Serum triglycerides values at 0, +3, +6 and +12 months were recorded. In majority of cases an increase in triglyceride level was noted at +3, +6 and +12 months but the difference was found to be statistically insignificant.

Multicentric trial of centchroman (30 mg weekly dose) carried out by C.D.R.I. Lucknow also showed that centchroman has no effect on serum lipid profile in human volunteers.

Ovarian size :

Ovarian size was ultrasonographically measured at 0, +3, +6 and +12 months of centchroman use. Ovarian size was expressed as ovarian volume which was calculated using the ellipsoid formula.

Ovarian volume = L x A P x T x 0.5 cc.

L = Longitudinal axis

A P = Anteroposterior axis

T = Transverse axis

The size of the ovary may vary according to the patient's menstrual cycle and endocrinologic status. Volumetric determinations are helpful in discriminating normal from enlarged ovaries. Fleischer considers normal ovarian volume to be approximately 10 cc. Nicolini (1985) considers

an ovary to be pathologically enlarged if the ovarian volume is more than 15 cc. Munn et al (1985) did a study on ultrasonographic determination of ovarian volume. They found the mean ovarian volume to be 6.48 cc ± 2.90 (Range 2.15 - 13.84 cc).

In our study at 0 month the smallest ovarian volume noted was 2.92 cc and the highest value was 10.06 with a mean ovarian volume of 4.96 cc which was well within the accepted limits.

The 0 month ovarian volume (a measurement of ovarian size) was compared with +3, +6 and +12 months ovarian volume. At +3 months a rise in ovarian volume was noted in 8 out of 9 patients and one patient showed a decrease in ovarian volume. The rise was statistically insignificant.

All the 10 patients at +6 months showed an increase in ovarian volume when compared with initial (0) values and this rise was found to be statistically significant ($p < 0.01$).

Comparison of 0 and +12 values also showed an increase in ovarian volume at +12 months but this rise was again not significant.

Thus an insignificant rise in ovarian volume at +3 months and a statistically significant rise at +6 months was noted. At +12 months the ovarian volume was found to decrease again in comparison to +3 values, but the values were still higher than initial (0) values. That is to say the difference (increase) in ovarian volume at +12 months and 0 months of use was statistically insignificant.

Hence with use of centchroman an increase in ovarian size at +6 months was noted. It reverted back to normal at +12 months.

Vaidya et al (1977) in their study on activity profile of centchroman in healthy female volunteers attributed the increase in cycle length to lengthening of follicular phase. They concluded that the plasma total estrogens are increased due to increased steroidogenesis by the ovaries. This could be due to stimulatory effect of the drug on hypothalamopituitory ovarian axis or due to direct action of centchroman on ovary sensitizing it to circulating estrogens.

Increase in ovarian size could be explained on the basis of delayed ovulation due to prolonged follicular phase. Due to prolonged ovarian stimulation the unruptured follicle could lead to cystic change in ovary, being interpreted as increased ovarian size. But the decrease in ovarian size from +6 to +12 months could not be explained on this basis.

An increase in ovarian size at +6 months followed by a decrease at +12 months could be directly somehow related to the effect of the drug centchroman.

Karyopyknotic Index :

During the treatment cycles a decrease in karyopyknotic index (KPI) on 14th day was noted as compared with pretreatment cycle. KPI on 21st day in treatment cycles also showed a decrease when compared with initial (0) cycle KPI on 21st day.

This pattern of decrease in KPI is a reflection of the antiestrogenic property of centchroman.

Rama Vaidya et al (1977) demonstrated a distinct antiestrogenic effect of centchroman on KPI at 120 mg/week and 60 mg/week dose leve. It was pointed out that the antiestrogenic effect could be variable depending upon the target sites like vagina, cervix and uterus.

Munshi et al (1977) demonstrated antiestrogenic effect of centchroman at 120 mg dose schedule in the form of depressed KPI by vaginal cytology inspite of markedly increased circulating total estrogens.

Changes in maturation index were parallel to those in KPI. With decreasing KPI a fall in the percentage of superficial cells and corresponding increase in percentage of intermediate cells was noted.

Overall in 86.76% cycles a decrease in KPI was noted on 21st day of treatment cycle as compared with KPI on 14th day. Thus in 86.76% cases there was evidence of progestogenic effect on 21st day of cycle. In 13.24% cycles (9 in number) lack of progestogenic effect evidenced by increase in KPI on 21st day was noted.

Out of these 9 cycles 66.67% (6 cycles) were prolonged and hence increase in KPI could be due to prolongation of the follicular phase, leading to persisting estrogenic effect even on 21st day. In the other words there was a lack of progestogenic effect.

In the rest 33.33% (3 cycles) the cycles were of normal duration and persistence of estrogenic effect on KPI even on 21st day could be due to anovulation and hence lack of progestogenic effect.

Spinbarkeit Test :

Spinbarkeit test on 14th day of treatment cycles showed decreased length of thread formation. This reflects the antiestrogenic effect. On 21st day also the length of the thread formed was decreased, again reflecting the antiestrogenic effect. Rama Vaidya et al (1977) noted the antiestrogenic effect on cervical mucous also at 120 mg/week dose. At 60 mg/week dose cervical mucous was found to show improvement.

Out of 68 cycles studied 76.47% cycles had a decrease in spinbarkeit at 21 days when compared with that at 14 days. Thus a progestogenic effect could be demonstrated on 21st day.

Here again 9 cycles showed increase in spinbarkeit on 21st day. Out of these 77.78% cycles were prolonged leading to persistence of estrogenic effect even on 21st day. 22.22% cycles were of normal duration and here lack of progestogenic effect could be due to failure of ovulation.

Fern Test :

Ferning was also found to be reduced in treatment cycles on 14th day. No consistent pattern was found on 21st day.

Out of 67 cycles studied 86.57% cycles demonstrated a progestogenic effect whereas the remaining 9 cycles showed lack of progestogenic effect. Out of these nine 77.78% cycles were prolonged and 22.22% cycles had normal cycle duration.

Taking MPI, Ferning and Spinbarkeit test as an indirect indicator of ovulation 83.27% cycles provided indirect evidence of ovulation.

1981 Annual Report of C.D.R.I. mentions that the maximum cervical score in the pretreatment, treatment and posttreatment cycle is similar. Only the day of the peak score is shifted according to duration of the cycle.

Vaidya et al (1977) studied the activity profile of centchroman and deduced that centchroman at 120 and 60 mg/week dose does not seem to inhibit ovulation, although it may be delayed. The contraceptive effect was thought to be mainly owing to its action on cervical mucous and endometrium affecting sperm transport and implantation.

Side effects :

The side effects noted in the present study were prolonged cycle, short cycle, scanty menses, menorrhagia and reduced appetite. There was not definite pattern of their ocurrence.

S.N. Roy et al (1977) found similar symptoms in the groups receiving placebo as well as the drug.

Thus centchroman was found to be well tolerated by human beings.

Failures :

Out of the 10 patients studied only one patient conceived after registration. She was a patient failure, the pregnancy ocurring after missing few tablets. She decided to continue with her pregnancy.

SUMMARY AND CONCLUSIONS

SUMMARY AND CONCLUSIONS

The present work was done in reproductive age group females; to study the effect of a new non steroidal oral contraceptive CENTCHROMAN on ovulation and ovarian size and various biochemical parameters. A total of 18 patients were studied.

Ovarian size measurement was done by ultrasonography at initial pretreatment month and +3, +6 and +12 months of treatment. At similar time period the biochemical tests were also carried out.

For ovulation three parameters namely vaginal cytology and cervical mucous for ferning and spinbarkeit formation were considered. These three parameters were studied in initial pretreatment cycle on 14th and 21st day and then subsequently in every treatment cycle.

Centchroman was given according to 30 mg biweekly schedule for the first 3 months and then 30 mg once a week for the rest of the time. First tablet was taken on the first day of menses and subsequent tablets on every Sunday and Wednesday irrespective of the menses day or delayed menstruation. From the 4th month onwards patients were asked to take one tablet on every Sunday irrespective of the menses day.

Conclusion :

Following conclusions were drawn from the present study :

1- Volunteers selected for the trial were 20-35 years of age; with a parity of 1-5 or more. Maximum patients were in the age group 23-25 and 26-28 years with a parity of two.

2- Centchroman causes delayed menstruation. Menstrual delay upto 45 days was found in 10.71% cycles of use and more than 45 days in 4.46% cycles.

Prolongation of cycles was neither restricted to any individual nor it showed any consistent pattern hence it may be difficult to attribute this effect to centchroman.

3- Various biochemical tests (urinalysis, haematological tests, lipid profile) were within normal limits. Only a significant fall ($p < 0.05$) in serum bilirubin level was noted at +6 months of drug use.

4- Centchroman caused a significant increase ($p < 0.01$) in ovarian size at 6 months of drug use and this reverted back to normal at 12 months.

5- A fall in MPI on 14th and 21st day in various treatment cycle in comparison to initial cycle was noted demonstrating the antiestrogenic effect of centchroman.

6- Cervical mucous study revealed decreased spinnability in treatment cycles on 14th and 21st day.

Decreased ferning was observed on 14th day but no consistent pattern found on 21st day of treatment cycles again revealing the antiestrogenic effect.

7- Taking KPI, spinbarkeit and ferning as indicator of ovulation 83.27% cycles were found to be ovulatory.

8- The side effects observed were menstrual disturbances and reduced appetite. The menstrual disturbances were in the form of prolonged cycle, scanty menses, menorrhagia and short cycles.

9- Only one patient failure was found. None of the volunteers had method failure.

The antiestrogenic property of centchroman could be responsible for its antifertility action. The contraceptive effect may be owing to its action on cervical mucous; making it impenetrable for sperms; and endometrium affecting implantation of fertilized ovum.

It was difficult to draw a definite conclusion from the study because of limited number of patients and improper follow up.

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